J. Cell Sci. **10**, 683–691 (1972)
Printed in Great Britain

A NEW JUNCTIONAL STRUCTURE IN THE EPITHELIA OF INSECTS OF THE ORDER DICTYOPTERA

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SUMMARY

The junctional complexes in the epithelia of insects of the order Dictyoptera have been investigated using the freeze-etch technique. As well as septate junctions, a new type of junction has been identified and the name 'inverted gap junction' proposed. The patch-like distribution of the inverted gap junctions basal to and often closely associated with septate junctions is very similar to the form of gap junctions and their relationship to tight junctions in vertebrates. This suggests that the inverted gap junctions, like normal gap junctions, could perform a communicating function between epithelial cells. The following features distinguish inverted gap junctions from normal gap junctions in freeze-etch preparations: (i) the arrays of particles and holes within inverted gap junctions appear on B- and A-type faces respectively, i.e. on the opposite faces to the particles and holes in gap junctions; (ii) the particles within inverted gap junctions appear to lie in rows which anastomose to form an irregular net, and not in an hexagonal array, as occurs in gap junctions.

INTRODUCTION

Only a limited number of different types of cellular junctions have been identified between epithelial cells. Some of these, i.e. septate junctions (Wood, 1959; Gilula, Branton & Satir, 1970; Flower, 1970, 1971), tight junctions (Farquhar & Palade, 1963; Staehelin, Mukherjee & Williams, 1969; Goodenough & Revel, 1970) and desmosomes (Farquhar & Palade, 1963; Kelly & Luft, 1966) appear to function in intercellular adherence, the first 2 normally being found as the outermost component of the junctional complex in invertebrate and vertebrate epithelial tissue respectively. Only one type of junction, the gap (or close) junction, has been specifically identified as a communicating junction between epithelial cells (Goodenough & Revel, 1970; Revel & Karnovsky, 1967), and until recently this junction had not been reported in invertebrate epithelia. However, lately Hagopian (1970), Hudspeth & Revel (1971) and Reger (1970) have all observed junctions in invertebrate epithelia which resembled gap junctions in their cross-sectional appearance, while Flower (1971), utilizing freeze-etching, has identified gap junctions in a mollusc.

The present study was undertaken to investigate the presence of junctions other than septate in invertebrates. It utilizes the freeze-etching technique to show that, as well as septate junctions, a completely new type of junction is present between epithelial cells in insects of the order Dictyoptera.

MATERIALS AND METHODS

Specimens were obtained from the accessory colleterial glands of the mantis, *Orthodera ministralis*, and the cockroach, *Periplaneta americana*. These glands were excised and soaked for about 1 h in 25 % glycerol in phosphate buffer at pH 7·4. Small samples were then dissected out and frozen in Freon 12 at −150 °C. Freeze-etching was carried out as described by Moor & Muhlethaler (1963) on a Balzers BA 500. Replicas were examined in a Philips EM 200 electron microscope.

RESULTS

When a membrane is fractured during freeze-etching it is split internally. This reveals 2 new faces, in freeze-etch replicas, which were originally apposed to one another within the membrane. It is proposed to label these faces as outlined by McNutt & Weinstein (1970). Thus the face of the membrane fragment which is still attached to the cell cytoplasm will be labelled face A and the opposite fracture face, face B. This system allows the 2 true surfaces of the membrane, which are normally observed only after deep etching to be labelled C and D.

The protein-secreting cells of the accessory colleterial glands in both mantis and cockroach are in the form of a single layer of cells lining the tube-like lumen of the gland (Kenchington & Flower, 1969; Mercer & Brunet, 1959). During freeze-etching of these glands the fracture plane occasionally runs along the boundary between two cells and out into the gland lumen. Whenever this occurs septate junctions can be seen running parallel to the epithelial, or luminal, surface of the cells (Fig. 1). Close examination of this and other similar micrographs shows that it represents an A face (*a*) and that on this face the septate junctions appear as rows of particles. Thus the particles in these septate junctions appear on the same membrane face as those previously described in freeze-etch studies of other invertebrate epithelia (Flower, 1970, 1971; Gilula *et al.* 1970).

A further type of junction is present in dictyopteran epithelia basal to the septate junctions. This can be recognized on A-type plasma membrane faces as discrete patches raised slightly above the general level of the membrane (*ij* in Figs. 1 and 2). It is proposed to use the name 'inverted gap junction' to describe this new type of junction; the reasons for using this nomenclature will be explained in the Discussion. When the inverted gap junctions are examined at higher magnification (Fig. 2), and their shadowed appearance is compared with that of the particles in the neighbouring septate junctions, it can be seen that a dense array of holes is present on their raised surfaces. These holes have a minimum centre-to-centre separation of 10 nm and do not appear to be regularly arrayed, as are the holes in gap junctions (Chalcroft & Bullivant, 1970; McNutt & Weinstein, 1970). In many replicas the inverted gap junctions appear to be surrounded, or nearly surrounded, by septate junctions.

Occasionally the fracture plane passes from one plasma membrane to the other within the junctional structure (Fig. 3). When this occurs the upper-lying plasma membrane face can be identified as a B face and the lower-lying one as an A face. On the A face (Fig. 3) the junction is again characterized by an array of holes as in Fig. 2,

while on the B face (*b*) an array of particles each approximately 8 nm in diameter is present. Although single particles can be identified on this face of the junction they mostly appear to lie in rows in which individuals cannot readily be seen. The particle rows in turn appear to anastomose to form an irregular network.

In many replicas, the structures on the 2 faces of inverted gap junctions are not so easily identifiable (Fig. 4). This could be due either to a change in the shadowing angle, or to a variation in the density of the structure within the junction. However, by comparing the appearance of the 2 faces of the inverted gap junctions in Fig. 4 with those of the junction in Fig. 3 it is obvious that once again a network consisting of rows of particles is present on the B faces, and that this network encloses islands of undifferentiated membrane face. Similarly, comparison of the A faces in the 3 micrographs suggests that rows of holes, or grooves, which also delineate plaque-like regions of undifferentiated membrane face are present on these faces in Fig. 4. Thus the A and B faces of the junction appear to present complementary structures in the sense that an A face appears to have been fractured away from a B face and vice versa. Therefore, unlike gap junctions in vertebrates (Chalcroft & Bullivant, 1970; Revel & Karnovsky, 1967) or invertebrates (Flower, 1971), inverted gap junctions do not display regularly arrayed particles or depressions on the 2 membrane faces revealed by freeze-etching. As in gap junctions, however, the separation of the 2 plasma membranes decreases rapidly near the junction (Fig. 4), both plasma membranes bulging out into the intercellular space so that in the junctional region the 2 plasma membranes are closely apposed.

DISCUSSION

It now seems to be generally agreed that membranes fracture internally during freeze-etching (Branton, 1966, 1967; Chalcroft & Bullivant, 1970; Sleytr, 1970; Wehrli, Mühlethaler & Moor, 1970). Thus it would be expected that the 2 faces formed during fracturing of an individual membrane would be complementary. This normally occurs in such a way that the majority of the particles present in the membrane appear on the A-type fracture faces. Although the B-type fracture faces rarely have sufficient holes in them to be the exact converse of the A-type faces, they do usually have many fewer particles on them. Such an asymmetric distribution of particles on the 2 fracture faces is also normally found in freeze-etch replicas of junctional structures. Thus the particles of both septate junctions (Flower, 1970, 1971; Gilula *et al.* 1970) and gap junctions (Chalcroft & Bullivant, 1970; McNutt & Weinstein, 1970) and the 'fibres' of tight junctions (Staehelin *et al.* 1969; Goodenough & Revel, 1970) are all normally located on A-type membrane faces.

For such an asymmetric distribution of particles to occur consistently most particles within membranes must be fastened more tightly in some way to the half of the membrane nearest to the cytoplasm than to the outer half of the membrane. This general rule applying to the attachment of particles within membranes is not followed by inverted gap junctions. In freeze-etch replicas of these junctions the particles appear on B-type fracture faces and the equivalent array of holes on A-type faces. This inversion

in the position of the particles is particularly evident in Fig. 2, where the particles of the septate junction are on the same plasma membrane face as are the holes in the inverted gap junctions.

The present investigation has shown that the inverted gap junctions are located basal to, and often surrounded by, septate junctions and are present as discrete patches. They therefore bear a general similarity in shape and position to gap junctions described in other tissues. The main differences between gap junctions and inverted gap junctions are, first, that the arrays of particles and holes within the inverted gap junctions appear on B- and A-type faces respectively, i.e. on the opposite faces to the particles and holes in gap junctions, and secondly, that the particles within the inverted gap junctions appear to lie in rows which anastomose to form an irregular net and not in an hexagonal array as occurs in gap junctions. That this difference in structure between gap and inverted gap junctions is not due to a variation in the freeze-etch technique is indicated by the fact that the present author, using identical preparation methods, has found 'normal' gap junctions in other invertebrates, namely the molluscs (Flower, 1971).

A sectioning study of the junctions occurring between epithelial cells in another dictyopteran, the cockroach *Leucophaea maderae*, has been carried out by Hagopian (1970). The presence of both septate junctions and junctions having the uniform 2–3 nm spacing between membranes typical of gap junctions was reported in this investigation. However, in the present freeze-etch study of epithelial cells from 2 other Dictyoptera typical gap junctions have not been identified, only septate junctions and the inverted gap junctions described in this paper having been found.

Because of the close relationships between the insects studied it would seem to be more than probable that the junctions observed by Hagopian (1970) were in fact the inverted gap junctions described in this paper. Since the junctions shown by Hagopian (1970) appear to be identical to vertebrate gap junctions in sections (see McNutt & Weinstein, 1970) when fixed and stained by similar procedures, it is possible that only the internal membrane structure varies between the gap and inverted gap type junctions. If this were so the membranes of inverted gap junctions could be held 2–3 nm apart exactly as in vertebrate gap junctions, so giving the 2 types of junction identical appearance in sections. The difference in the arrangement of particles within the membranes could then give the different freeze-etch appearances of the 2 types of junction. Because of these similarities in the 2 types of junction and yet the need to denote their very different appearances in freeze-etch replicas, the name 'inverted gap junction' is proposed for the new type of junction identified in Dictyoptera during the present investigation.

I should like to thank Mrs S. M. O'Kane and Mr L. M. Adamson for expert technical assistance and Messrs W. S. Bertaud and D. M. Hall for helpful discussion.

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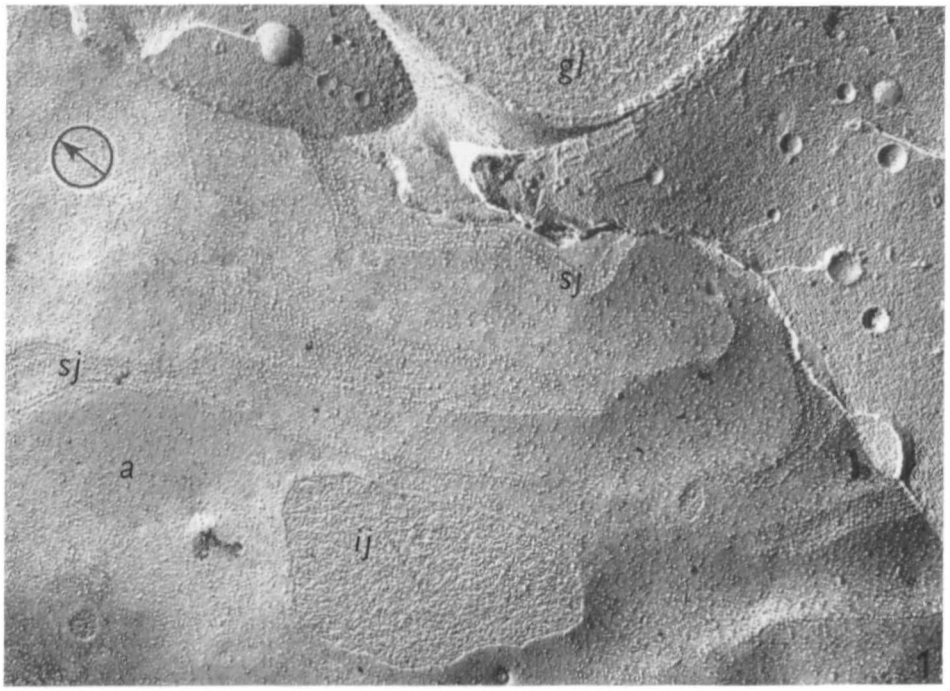
(*Received* 1 *September* 1971)

Note: The shadowing direction has been indicated by a circled arrow in all freeze-etch replicas.

Fig. 1. A cell membrane adjacent to the gland lumen (*gl*) has been revealed in this replica. Septate junctions (*sj*) can be identified as rows of particles running close to the end apparatus. A further possible differentiation (inverted gap junction) of the plasma membrane (*ij*) is apparent basal to the septate junction. *a*, A face. × 38000.

Fig. 2. Higher magnification view of septate (*sj*) and inverted gap (*ij*) junctions. Note that the white 'shadows' are towards the top of the micrograph on the particles of the septate junction, whereas they are towards the bottom of the micrograph in the inverted gap junctions. This indicates that on this plasma membrane face the inverted gap junctions are present as an array of holes. Whereas the septate junctions appear to consist of long, or continuous, rows of particles which surround the entire epithelial surface of the cell, inverted gap junctions are present in the form of discrete patches. × 50000.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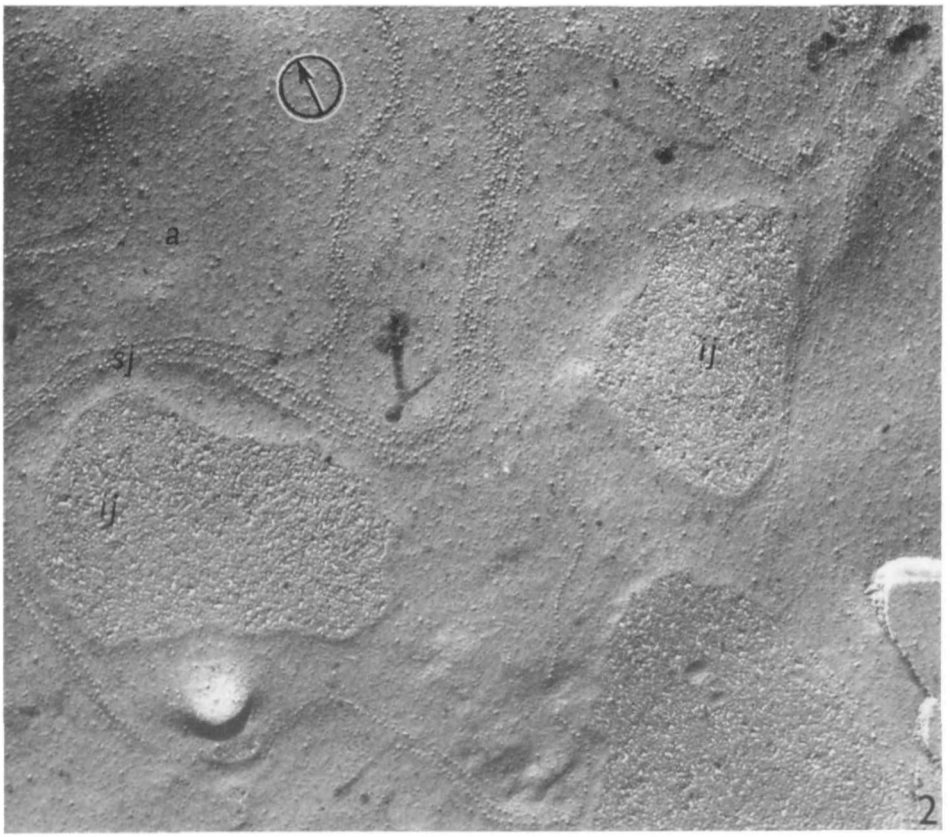
1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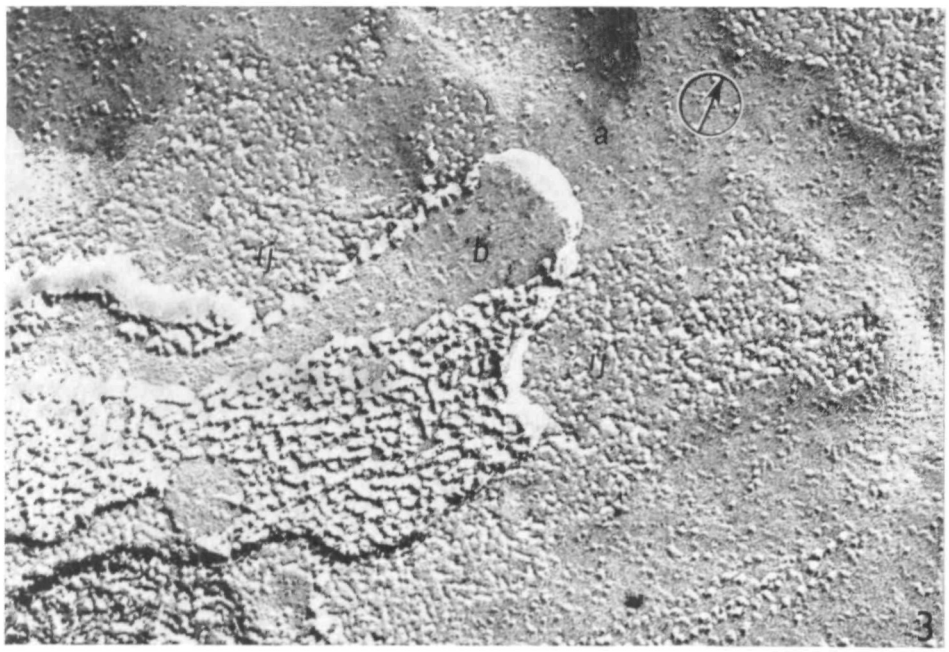
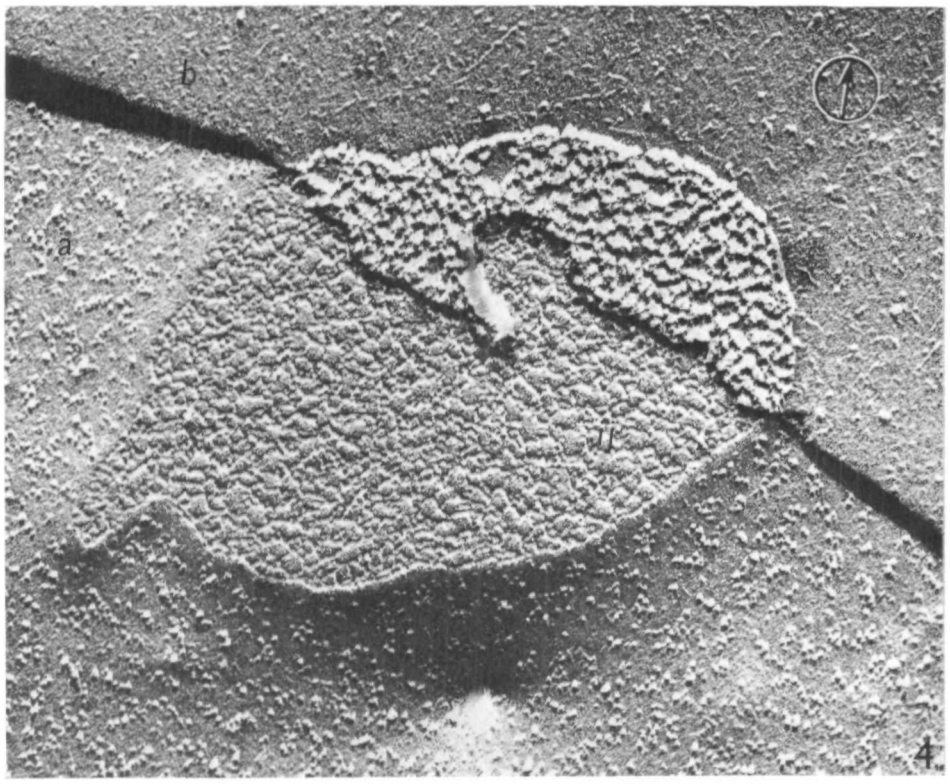
2

Fig. 3. Replica in which the fracture plane has jumped from one plasma membrane to the other within an inverted gap junction (*ij*). The lower-lying, or A-type plasma membrane face (*a*) contains a denser array of particles than the upper-lying, or B-type plasma membrane face (*b*), as is found on most membranes. In the inverted gap junction, however, the particles are on the B face and the array of holes on the A face. Individual particles can be seen on the B face of the junction. × 100000.

Fig. 4. Micrograph showing another inverted gap junction (*ij*). By comparison with Fig. 3 the A face can be seen to consist of rows of particles and the B face of grooves, or rows of holes. The networks of rows of holes and particles on the 2 faces both delineate areas of undifferentiated plasma membrane. The shape of the shadows caused by the step down from one plasma membrane to the next indicates that the plasma membranes become closely apposed within these junctions. × 80000.


a
b
3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a
4