ARM-BEARING MICROTUBULES ASSOCIATED WITH AN UNUSUAL DESMOSOME-LIKE JUNCTION

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INTRODUCTION

Microtubule fine structure is well documented in many diverse cell types (see reviews by Porter, 1966; Schmitt and Samson, 1969). In most instances a typical 200–250 A diameter structure is described. Occasionally, additional characteristics such as arms or bridges are seen in cells other than typical flagella and cilia. Selected examples of these include: frog neurotubules (Kohno, 1964); axostyle microtubules (Grimstone and Cleveland, 1965); tentacle microtubules (Rudzinska, 1965); spindle microtubules (Krishan and Buck, 1965); intranuclear microtubules (Behnke and Forer, 1966); sperm microtubules (Robison, 1966); axopodial microtubules (MacDonald and Kitching, 1967); spermatid microtubules (McIntosh and Porter, 1967); pyramidal cell neurotubules (Palay, 1968); groupings of microtubules in several ciliate species (Tucker, 1968; Bannister and Tatchell, 1968); plant cell plates (Hepler and Jackson, 1968); algal spindle fibers (Wilson, 1969); dendritic neurotubules (Wuerker and Palay, 1969); insect sensory cell microtubules (Smith, 1969); heliozoan microtubules (Tilney and Byers, 1969); tissue culture cells (Hepler, McIntosh, and Cleland, 1970); heliozoan microtubules (Roth, Pihlaja, and Shigenaka, 1970).

Likewise, the fine structure of junctional complexes has been well documented (Farquhar and Palade, 1963, 1965; Kelly, 1966). Generally, these are divided into typical desmosomes (maculae adherentes), zonulae adherentes, and the zonulae occludentes. In addition, the septate desmosome has been described (Locke, 1965; Gouranton, 1967; Bullivant and Loewenstein, 1968).

However, the number of descriptions of junctional complexes associated with microtubules is only a very small proportion of the total reports. In 1966, Noirot-Timothée and Noirot described the insertion of microtubules in the plasma membrane of foregut and hindgut epithelial cells of termites. These junctional areas or "attachment zones" were obscured by dense material adjacent to the internal lamella of the membrane. Lai-Fook (1967), in describing developing muscle insertions in insects, stated that the microtubules of the tendinous epidermal cells did "not merely approach the membrane but appeared to attach to it at the site of intermediate junctions and hemidesmosomes."

Moulins (1968) also described microtubules inserting on plasma membranes of epithelial cells in *Blabera craniifer*.

The present study provides some additional observations on arm-containing microtubules and their association with and participation in an unusual junctional complex.

MATERIALS AND METHODS

Tibias of the first pair of legs of the adult cricket, *Gryllus assimilis*, were removed in entirety by sectioning proximal to the knee joint and distally above the tarsus. The tibias were routinely fixed in phosphate-buffered (Clark, 1963) glutaraldehyde (Sabatini,

Bensch, and Barrnett, 1963) and osmium tetroxide, dehydrated, and oriented for longitudinal and transverse sectioning while being embedded in Epon 812 (Luft, 1961). Alternating serial sections were cut at 1½ and ¼₀ μ from each tibia with a diamond knife fitted to a Porter Blum MT-1 ultramicrotome. For light microscopy, 1½-μ thick sections were stained with toluidine blue (Trump, Smuckler, and Benditt, 1961). Ultrathin sections were stained on the grid with uranyl acetate and lead citrate (Reynolds, 1963; Venable and Coggeshall, 1965) and examined with an RCA EMU-3F electron microscope.

RESULTS

In a previous investigation (in preparation for press), it was shown that the interstitial cells of the subgenual and intermediate sensory organs of the cricket foreleg tibia are unique in several respects. At least two cell types, designated light and dark cells, are distinguishable solely on the basis of the concentration of microtubules present. Furthermore, interstitial cells of these scolopophorous sensory organs are unique in the very unusual type of junctional complex associated with them. These are broad desmosome-like interconnections, indicated by *DLJ* and the encircled asterisk (⊛) in Figs. 1 and 2. When seen in longitudinal and tangential profiles, the length of these junctions varies considerably but may be up to 8 μ long. In both the subgenual and intermediate sensory organs, the nature of the relationship between the desmosome-like junction (*DLJ*, Figs. 1 and 2) and the microtubules is similar. The microtubules are scattered throughout the fibrillar and granular cytoplasm along with occasional mitochondria. The desmosome-like junctions seemingly maintain a typical intercellular distance of 80–100 A, in that marked dilatations of the extracellular space usually are seen only in areas where the desmosome-like junctions are absent. (*EX*, Figs. 2, 3)

The microtubules, about 200–220 A in diameter, are oriented parallel to the junctional membranes at a distance of about 40 A from the inner lamella of the membrane. The microtubules are interconnected by arm-like structures (arrows, Fig. 3) which are about 125–135 A in length and 40–50 A in width.

The arms, which extend to the inner lamella of the membrane, may form angles as great as 60° incidental to the membrane or may just parallel the membrane.

The number of microtubules within a desmosome-like junction varies from about 4 to 10, the majority of the junctions having around seven microtubules associated with each membrane.

The microtubules that contribute to the desmosome-like junction are similar in size to those present in the cytoplasm of the interstitial cells. Arm-bearing cytoplasmic microtubules not associated with the desmosome-like junction are often seen in either rosette or linear configurations (Fig. 4).

DISCUSSION

Only a few reports have described a close association between microtubules and membranes (Smith, 1969), or even "attachment" of microtubules to membranes (Moulins, 1968; Noirot-Timothée and Noirot, 1966; Lai-Fook, 1967). In most examples, a region of high electron opacity exists in the attachment zone thereby obscuring junctional detail. To the author's knowledge, there have been no reports of junctional complexes associated with arm-bearing microtubules such as are seen in the desmosome-like junction described here.

The interpretation of the functional significance of arm-bearing microtubules has been the subject of numerous hypothetical discussions. Since the discovery that an ATPase protein (called dynein by Gibbons and Rowe, 1965) exists as a part of the arm structure in cilia of *Tetrahymena*, many structural-functional relationships have been theorized. A good summary of most of the current literature, including that on microtubule protein chemistry, is presented in Schmitt and Samson's review (1969). Suffice it to say that, from data presented here, it is not possible to interpret the functional significance of this junctional complex. It is interesting to note that the interstitial cells, where the desmosome-like junctions are found, are integral parts of the mechanotransducing systems of the subgenual and intermediate organs. In this regard they are sensitive to vibrations, either from direct transmission through the cuticle to which they are attached, or via the circulating hemolymph in which they are suspended. Since this same hemolymph fills the extracellular areas between the interstitial cells, the desmosome-like junctions may function in an active manner in the transducing mechanism itself, as well as in the maintenance of morphological integrity.

Other descriptions and further implications will be discussed in work presently in preparation.

SUMMARY

The purpose of this report is to present some observations on arm-containing microtubules and their association with and participation in an unusual junctional complex. Foreleg tibias of the adult cricket, *Gryllus assimilis*, were removed and prepared routinely for electron microscopy. Examination of the interstitial cells of the subgenual and intermediate sensory organs located within the tibias revealed the presence of arm-bearing microtubules associated with desmosome-like junctions. The microtubules, about 200–220 A in diameter, are oriented parallel to the junctional membranes at a distance of about 40 A from the inner lamella of the membrane. The microtubules are interconnected by arm-like structures which are about 125–135 A in length and 40–50 A in width. Arm-bearing cytoplasmic microtubules not associated with the desmosome-like junctions are often seen in either rosette or linear configurations. The desmosome-like junctions may function in an active manner in the transducing mechanism of the subgenual and intermediate sensory organs, as well as in the maintenance of morphological integrity.

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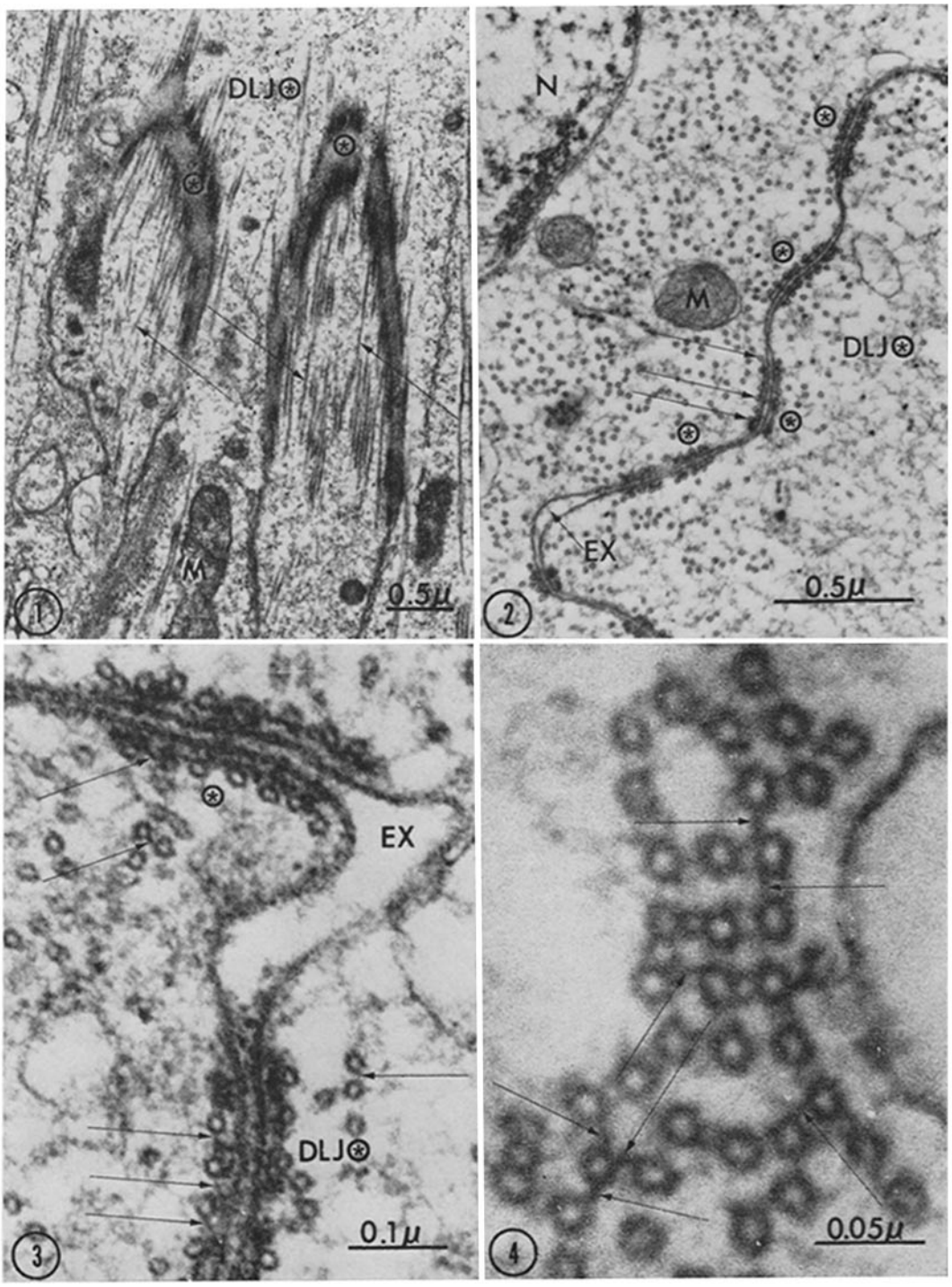
FIGURE 1 Oblique section through portions of interstitial cells of the intermediate sensory organ found in the foreleg tibia of the cricket, *Gryllus assimilis*. Broad desmosome-like junctions (*DLJ*, ⊛) are seen at the junctions of the cells. Several mitochondria (*M*) and numerous microtubules (arrows) are present. × 19,400.

FIGURE 2 Transverse section through portions of interstitial cells of the intermediate sensory organ found in the foreleg tibia of the cricket, *Gryllus assimilis*. Desmosome-like junctions (*DLJ*, ⊛), nucleus (*N*), extracellular space (*EX*), mitochondrion (*M*) are present. Microtubules (arrows) are seen forming the junctions and are also present within the cytoplasm. × 39,800.

FIGURE 3 Transverse section through portions of two interstitial cells of the intermediate sensory organ found in the foreleg tibia of the cricket, *Gryllus assimilis*. Two desmosome-like junctions are present (*DLJ*, ⊛). Extracellular space (*EX*). Arms (arrows) parallel the membranes. × 140,500.

FIGURE 4 A group of arm-bearing microtubules is seen within the cytoplasm of a similar epithelial cell. The arms (arrows) appear to link the microtubules. × 313,500.



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