MICROTUBULES AND MICROFIBRILS
IN MORPHOGENESIS OF THE SCALE
CELLS OF EPEHSTIA KÜHNIELLA

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ABSTRACT
The development of scale cells in insects has been studied from the appearance of the first cytoplasmic projection which forms the scale rudiment. This rudiment contains numerous longitudinally oriented microtubules throughout. Immediately under its outer surface lie a series of adjacent but distinct bundles of longitudinally oriented circa 60-A fibrils with a circa 120-A center-to-center spacing. As the rudiment broadens, the microtubules become distributed near the surface. The rudiment finally becomes extremely broad and flattened. Fibril bundles are now widely separated and equally spaced. They still lie immediately below the cell surface. Then the cytoplasm protrudes midway between each fibril bundle to form longitudinal ridges and the major shape changes of the scale have been achieved. The final pattern can thus be related to the cytoplasmic organization of the rudiment. The main cytoplasmic elements which seem important in scale morphogenesis, on the basis of frequency, orientation and grouping, are 60-A fibrils and microtubules.
changes. Microtubules are very numerous in the outgrowing scale, and their spatial arrangement changes with the early changes in scale shape. Bundles of longitudinal fibrils are closely juxtaposed under the outer surface of the first projection. They become widely separated as the developing scale flattens, and the ridge and rib pattern which develops is spatially related to them.

MATERIALS AND METHODS

_Ephestia kuhniella_ wild type stock R1345E obtained from Dr. Jane Shoup was raised on yellow corn meal at room temperature. Scale development was first studied by sampling early, mid, and late pupae, and later by placing prepupae at 19°C and allowing pupae to develop at this temperature so as to approximate the developmental time schedule used by other workers (22, 19). The central region of the wing was sampled in both fore and hind wings from pupae up to 19 days of pupation, after which the development of cuticle had become so extensive that it made preparation of the specimen impractical. Pupae were placed in chilled fixative where the wings were dissected out. Material was fixed in 6.25% glutaraldehyde (20), made up in phosphate buffer at pH 6.8, for 4 hr at 4°C. It was next rinsed in phosphate buffer at pH 7.6, then postfixed for 1 hr in 1% osmium tetroxide at pH 7.6. Tissues were run up through a graded series of alcohols in the cold, brought to room temperature, and embedded in Epon (15). Sections were cut with glass knives on an Porter-Blum MT-2 ultramicrotome and stained overnight in uranyl acetate. They were viewed with an RCA EMU3C electron microscope. Micrographs which were traced first printed at a magnification of 80,000 and the location of identifiable microtubules was recorded.

RESULTS

The first projection which occurs at the free cell surface is round in cross-section (Figs. 1 and 2). The outer rim as seen in similar view by light microscopy appears homogeneous (6, 22). This outer rim forms a band around the projection and is made up of longitudinally oriented fibrils, about 60 A, arranged with an approximately 120-A center-to-center spacing into bundles. The bundles are somewhat variable in size but in some regions they appear to be more regular and are about 1,000 A across. Each bundle is closely juxtaposed to the cell membrane which tends to be pushed out forming a scallop as seen in section. Microtubules about 250 A in diameter are numerous throughout the interior of the projection or scale rudiment. The quantity of microtubules is seen particularly well in longitudinal sections (Fig. 3) or in favorably oriented cross-sections (Fig. 4). Fig. 3 also illustrates the fact that microtubules are arranged predominantly parallel to the long axis of the projection proper (at the right of figure) and tend to be less numerous and less regularly oriented towards the cell body (at the left of figure). Thus both microtubules and fibril bundles have a longitudinal arrangement. The regular orientation and spacing of the fibrils is seen in an oblique cut (Fig. 5). Toward the distal tip of the scale rudiment, fibril bundles tend to continue into extensions from the cell surface (Figs. 7 to 9) often in an array corresponding in size and spacing to their regular arrangement in the rim of the projection. In favorable instances the fibrillar composition of these extensions is clear. Such extensions, described previously as pseudopodial (18), thus tend to be ordered by the spacing of fibrils in the cytoplasm.

The first change in shape which occurs is a broadening and flattening of the projection or future scale (Figs. 6 and 10). This occurs as the projection continues to increase in length and hence in bulk. The cytoplasm at this time is characteristic of an active cell in which abundant endoplasmic reticulum and Golgi bodies are present (Fig. 6). These structures occur towards the center of the developing scale. Microtubules now seem to occur predominantly toward the edge. This trend is illustrated in tracings of micrographs of scales which are progressively flattened (Fig. 10). Such a diagram, of course, includes only those microtubules which happen to be cut in cross-section, but it is also consistent with the impression derived from longitudinal sections and with the fact that microtubules have been described as occurring predominantly around the edge of a number of mature cells (21) and of maturing cells (3). If one examines the neck of the scale, long after the scale proper has flattened, one sees that where the original rounded contour of the scale remains a relatively regular distribution of microtubules occurs throughout the center, as is associated earlier with the rounded contour of the whole rudiment (Fig. 11). It may be noted that fibril bundles in this instance tend to be larger on the lower side of the scale. Such a difference in upper and lower surfaces has been observed in several instances and is reported by Pawelecz and Schlote (18) as the predominant arrangement.

The second major change in shape which occurs...
**FIGURE 1** The edge of an early projection of a scale cell. Bundles of fibrils (F) and microtubules (M) cut in cross-section. × 80,000.

**FIGURE 2** Cross-section of projection. Fiber bundles surround the entire edge. Compare with Fig. 7. × 37,000.

**FIGURE 3** Longitudinal section of the neck of a projection. Microtubules are more numerous in the projection proper at the right than in the lower part of the neck at the left which is connected with the cell body. × 32,000.
FIGURE 4  Cross-section of the neck of an early projection. Microtubules are prominent. The arms of the socket cell (S) extend only a short distance. Compare Figs. 21 and 11. $\times 32,000$.

FIGURE 5  Oblique cut at the edge of an early projection, showing the fibrils (F) cut in longitudinal section. $\times 64,000$.

FIGURE 6  A projection which is oval in cross-section. Microtubules are more numerous towards the surface. The interior shows endoplasmic reticulum, mitochondria, and Golgi apparatus (G). Compare with Fig. 10. $\times 32,000$. 

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is the development of longitudinal ridges. This process is illustrated in Figs. 12 to 14. These developing scales are greatly flattened. Microtubules are still present throughout the cytoplasm. They are longitudinally oriented but show no localized arrangement. With the widening of the scale, the distribution of fibril bundles has greatly altered. In early stages they were distinct but closely juxtaposed. Now they remain distinct but are widely separated. As the figures illustrate, they are still closely associated with the cell surface. The regular spacing of these longitudinal fibril bundles is correlated with a regular spacing of small protrusions (Figs. 12 and 13) each of which is midway between two bundles. These protrusions enlarge and then take on the modeling of the mature scale ridges. Under the conditions in which the pupae were maintained, the process of maturation illustrated in Figs. 12 to 14 required about a day. In those pupae sampled before day 10 no regular protrusions appeared, and in those sampled after this day the definitive outlines of the ridges were always present. Once the ridges have formed, the fibril bundles are very much less prominent, although they may be seen occasionally (Fig. 15).

A third form of change consists in the formation of fine ribs along the upper surface of the scale. The ribs originate at the base of the ridges and occur with very regular spacing and join adjacent ridges (Figs. 15 and 16). In oblique cuts through the scale, the origin of the ribs at the base of the ridges is clear. The very regular spacing of the ribs is evident, particularly in sections parallel to the flat surface of the scale (Fig. 16). Such sections permit a diagrammatic reconstruction of the scale (Fig. 17).

The diagram in Fig. 17 is the same as that of Kühn (8) which was derived from micrographs of whole scales, except that the contour of the ridge is shown with more accuracy in section. At this stage the moulding of the scale is essentially complete. It is noteworthy that the final contouring of the ridges and the rib formation appeared to occur simultaneously in a relatively short period during pupation. This change is associated with a more prominent appearance of the cell membrane as the outer layer of epicuticle, the cuticulin, is laid down (14).
to form the trabeculae which join the upper and lower surfaces. The ridges are solidly filled with an inner cuticular layer, which may be compared with that in the socket cell (Fig. 21). This part of the scale tends to look rather homogeneous after glutaraldehyde–osmium tetroxide fixation, as do the taenidia which back the ridges in trachea (2).

There are a number of different types of scales, some of which differ radically from those studied such as the bristlelike scales at the wing border. In the body of the wing, scales also differ in degree of polyploidy, size, shape, and timing of development (9). The general features of early development described here, however, appear to be similar for the covering, middle, and deep scales. Since the scales extend posteriorly and lie over one another, a sampling of ten to twenty scales may be obtained in the same micrograph (Fig. 22) and compared.

DISCUSSION

The scale rudiment elongates, flattens, and becomes ridged and ribbed. A descriptive study of the fine structure during these changes can give some clues as to possible underlying mechanisms.

Picken observed birefringence in the outer layer of the cytoplasm in the scale rudiment from the earliest recognizable stages of development. He suggested that fibrillar aggregates may cause elongation of the scales (19) and bristles (12). The fibril bundles located in this region form a framework for the rudiment and appear to extend distally beyond the main bulk of the cytoplasm but of course are not the only longitudinally oriented elements. Microtubules are in very plentiful occurrence, suggesting that their morphogenetic role is important. The early scale rudiment increases very rapidly in bulk. There are indications of synthetic activity, in that a very considerable amount of endoplasmic reticulum is present. The enlarging scale undergoes a systematic change in shape, which, it seems clear, is correlated with a change in the distribution of microtubules. Microtubules could serve a structural role, as is suggested by their distribution around the edge of the scale rudiment here as in other cells (3, 21), or they could initiate cytoplasmic movement, a possibility raised by Porter (3, 11). In the latter case, one might visualize a funneling of material from the cell body into the center of the growing rudiment. As the scale is flattened, the microtubules take on a fanlike arrangement. During this process, the fibril bundles which are at first closely juxtaposed become widely
Cross-section of the neck of a scale at about day 9 to day 10 of pupation. The neck of the scale is still round in cross-section and retains the distribution of microtubules and fibril bundles seen in the early projection. The neck is now surrounded by the socket cell. Scales are visible at the upper left. X 32,000. Insert, X 56,000.
Figures 12 to 14  Cross-sections of the flattened scale, which illustrate the development of longitudinal ridges at day 10 of pupation. Ridges develop as protrusions (R) on the dorsal and ventral surfaces of the scale. They form regularly between fiber bundles (F) which in early stages lie close together (see Fig. 1) and are now widely separated. All X 32,000.
Figure 15 Scales cut obliquely, showing attachments of ribs (Ri) to the ridges (R). Fiber bundles (F) are occasionally still visible. × 32,000.

Figure 16 Scale cut tangentially, showing ribs arising from two ridges. × 32,000.
separated. The bundles now have a regular spacing, becoming farther apart as they leave the neck region of the scale. It is conceivable that microtubules control this graded expansion. The surface is now closely underlaid by fibril bundles at regular intervals, and it is between these bundles and parallel to them that the cytoplasm bulges outward forming the first indication of the ridges. Taking into account the variations in scale size and type in the mature condition, one can say that the number of fibril bundles in an early stage, such as that pictured in Fig. 6, is roughly equivalent to the number of ridges to be expected in the finished scale. Thus, in part, the mature pattern can be said to be incipient from the beginning. It is interesting to note that the scale ridge pattern may be broken up into irregular patches by heat treatment of pupae at early stages (7) when the structures present to be affected must be the fibril bundles. This interpretation of the role of fibril bundles differs from that of Paweletz and Schlote (18) but does not appear to be inconsistent with their findings. These authors consider the fibril bundles to be cuticle precursors and stress the fact that the location of ridges does not coincide with that of the bundles. Their figures illustrate the regular occurrence of bundles along the lower surface, but show them clearly only occasionally on the upper surface. Ridges generally occur midway between bundles, as in the present work. Paweletz and Schlote consider it unclear whether the subcortical fibrils of the early rudiment are the same fibrils of which the bundles, which they see only later, are composed. Observations described here show that the subcortical fibrils consist of juxtaposed bundles. In general, the description of Paweletz and Schlote is confirmed here, and

**Figure 17** Diagrammatic reconstruction of a portion of a scale. Upper and lower surfaces are connected by trabeculae. In the mature scale, holes develop between the ribs and the interior becomes air filled. X 32,000.

**Figure 18** Oblique section cutting through 3 fiber bundles (F) and cutting across the forming ribs (Ri). Ribbed regions form near fiber bundles. X 32,000. Insert, X 48,000.
FIGURE 19 Cross-section of a scale at about 19 days of pupation. The cytoplasm has largely disappeared, leaving fibrous strands in the region of the trabeculae. The ridges resemble the inner layer of the cuticle as seen in Fig. 21. $\times$ 20,000.

FIGURE 20 Same as Fig. 19. $\times$ 100,000.

FIGURE 21 Cross-section through the neck of a scale of a late pupa. The cuticle forms a thick continuous layer. The two arms of the socket cell remain distinct and are juxtaposed (a) above the scale. The neck has become ridged. $\times$ 32,000.

FIGURE 22 Same as Fig 21. $\times$ 5,300.
any differences in detail might be easily accounted for by differences in preparative technique. The observations of the plenteous supply of microtubules and their distribution depend particularly on methods of preparation. Paweletz and Schlote stress the role of the cell membrane in initiating morphogenetic changes. A fuller description of the cytoplasmic organization of the rudiment emphasizes the fact that changes in contour of the cell membrane occur only in concert with a precise cytoarchitecture.

The next step in the process is the moulding of the ridges and the formation of the ribs. As the outward protrusions of the ridges form, a rounded trough results between each ridge (see Fig. 10). Locke has pointed out that “the elaborate scul
turing of the surface, the formation of hairs, scales, and microtrichia, and the elegant patterning of tracheae and tracheoles all takes place at an epidermal cell surface at the time of formation of the cuticulin layer. It is questionable which is the prime mover, the plasma membrane assuming a shape for the deposition of cuticulin or the cuticulin responding to forces which carry the plasma membrane along with it.” (14). The conformation of the ridges in the tracheae is such as to suggest that the pattern which is formed might be accounted for entirely as resulting from simple physical forces causing buckling of the expanding surface of an elastic cylinder. This results in a spiral or a series of annuli (13). In scales the rounded trough between two ridges forms a half cylinder, and conceivably the series of regularly spaced ribs formed here could arise in a similar way.

The foregoing discussion attempts to describe the various elements involved in scale formation and to link them in a logical, if very hypothetical sequence. The major form changes which the rudiment undergoes appear to be directly related to its early cytoplasmic composition. The importance of microtubules and microfibrils is indicated by their very plentiful occurrence, their orientation, and their grouping. These structures are widely distributed among many diverse cell types if not universally present, and both microtubules (3) and microfibrils (16, 17) have been related previously to morphogenetic changes in cells.

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