

## SPOKE HEADS IN SPERM TAIL OF *DROSOPHILA MELANOGASTER*

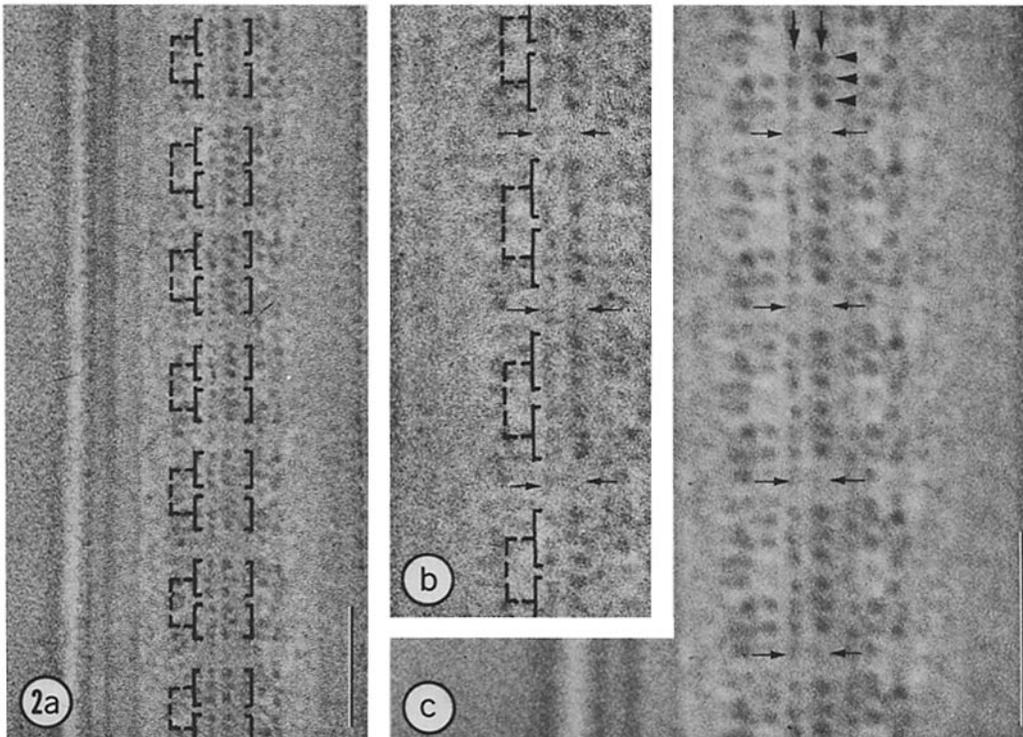
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Perotti (9), and Bairati and Perotti (4) showed in the axoneme of the sperm tail of *Drosophila melanogaster* that the innermost ends of the "spokes" of Afzelius (1) or "radial links" of Gibbons (6) are granular and that such granular structures are regularly arranged along the length of the axoneme. They also observed that short "projections" of the central tubules are arranged in a helical manner with a periodicity of 170 Å. Warner (15) revealed in the sperm tail of another dipteran insect, *Sarcophaga bullata*, that the central sheath which may correspond to the above

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FIGURE 1 Cross section of axoneme, viewed in the nucleus-to-tail direction. A pair of dots can be recognized at each spoke head. One at the counterclockwise position (arrowhead), which is closer to the center of axoneme (*O*) than the other (small arrow), is approximately in alignment with the centers of the axoneme and peripheral doublet, as indicated by a dotted line. Arrow *A* which connects a pair of dots is skewed counterclockwise against arrow *B* which is perpendicular to the broken line *OC* that connects the center of the axoneme and the midpoint of the pair. The no. 1 peripheral doublet is indicated by an asterisk on its *B* subtubule. *AT*, accessory tubule; *AS*, appendicle stripe (refer to [14] for its definition); *MM* and *mM*, major and minor mitochondrial derivatives; *PM*, plasma membrane. Bar represents 0.1  $\mu\text{m}$ .  $\times 360,000$ .

FIGURE 2 (*a*) Part of a longitudinal section of the axoneme. (*b*) Another part of the section at higher magnification. (*c*) A linearly translated superposition of a portion of the same structure (inherent period  $\times 7$ ). Six granules of about 60 Å width, which are arranged in two columns and three rows, form a group (parentheses in *a* and *b*). The pairs of groups (broken line parentheses) are arranged in a periodicity of approximately 900 Å, that allows identification of the groups as spoke heads. The columns (arrows in *c*) and rows (arrowheads) are separated by about 150 Å and 100 Å, respectively. In (*b*) and more clearly in (*c*), a pair of faintly defined granular structures (small arrows) is recognized between two pairs of spoke heads. Bars represent 0.1  $\mu\text{m}$ . (*a*)  $\times 160,000$ ; (*b*) and (*c*)  $\times 260,000$ .



projections has a periodicity of 160 Å, that a pair of links can be considered as a repeating unit, and that the highly ordered arrangement of such pairs is closely related to the periodicity of the central sheath. In the present study of the sperm tail of *D. melanogaster* in sections, new findings on the fine structures of the spoke head are described. The term spoke, which is older than radial link, is adopted here.

## MATERIALS AND METHODS

The methods used in this study are the same as those previously described in (14). Briefly, testes of *D. melanogaster* were dissected out in chilled 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, fixed for 2 h in fresh fixative, and postfixed for 2 h in 2% OsO<sub>4</sub> in 0.1 M phosphate buffer at 4°C. After ethanol dehydration, they were allowed to equilibrate with room temperature. Ethanol was then gradually replaced with propylene oxide and the testes were embedded in Epon 812. The Sorvall MT-2 ultramicrotome (Ivan Sorvall Inc., Norwalk, Conn.) was used for sectioning. Sections were doubly stained with uranyl acetate and lead citrate before being observed in a Philips EM-300 electron microscope.

In order to enhance the periodic structure in longitudinal section, the translational technique (inherent period  $\times$  5–7 translations) was used. Tail cross profiles presented in this paper are viewed in the nucleus-to-tail direction, which was adopted by Warner (15), as well as in our previous papers (13, 14).

## OBSERVATIONS AND DISCUSSION

The axoneme appears to be fully developed before the individualization of sperm from the syncytial condition (13, 14). The development of projections of B tubules of peripheral doublets to accessory tubules and further to satellites (5), with the addition of dense deposits, was studied by Kiefer (8) and can also be seen in the series of electron micrographs presented in our previous paper (13). The central singlet microtubules appear like ordinary cytoplasmic microtubules at early stages of spermiogenesis but develop into tubules of a complex profile (8, 13). Accessory tubules in the satellites of mature sperm tail are quite similar to the central tubules in appearance (2, 4, 9, 13; compare *AT* with the central tubules in Fig. 1). In the structure of the axoneme, sperm tails of many dipteran insects such as *Sarcophaga* (15), *Sepsis* (10), or *Ceratitis* (2) appear to be identical to those of *Drosophila*.

The spokes, which project from the A tubules of peripheral doublets toward the center of the axoneme, also develop during the preindividualization period, from vaguely defined structures to

conspicuous ones (8, 13). Their inner tips or heads are each seen as including two distinctly separated dense "dots" (small arrow and arrowhead in Fig. 1), as previously illustrated by Daems et al. (5). The dots are not clearly separated in some thick sections or when the staining is not adequately strong, which may account for the recognition of the head as a U-shaped structure by some workers (3, 15).

Precise knowledge of the arrangement of the spokes (15) permits an easy identification of the spoke heads in longitudinal sections of the axoneme. In a section which includes longitudinally aligned spoke heads of a peripheral doublet, each head is seen to contain two columns of three granules of about 60 Å in width (Fig. 2). The two columns are separated by about 150 Å and the three granules in a column by an interval of about 100 Å. These observations indicate that a dot seen in a cross section of ordinary thickness, i.e., 500–700 Å, represents a few superimposed granules rather than a single one.

When the two dots of each spoke head are viewed in the nucleus-to-tail direction, that in the counterclockwise position is always found to be closer to the center of the axoneme than the other (Fig. 1). In other words, nine pairs of dots are skewed counterclockwise, i.e., opposite to the skewing direction of the peripheral doublets, and the angle is estimated to be 15–20° (angle between arrows *A* and *B* in Fig. 1), appreciably greater than the skewing angle of the peripheral doublets, 10–15° (7, 15). Another feature of the arrangement of the dots or the columns of granules is that one of the paired dots which is at the counterclockwise position (arrowhead in Fig. 1) is approximately in alignment with the centers of the axoneme and the peripheral doublet (dotted line in Fig. 1).

The spoke head consisting of six granules and a matrix material of medium density around them is estimated to measure about 250 Å in width and about 300 Å in depth. In longitudinal sections of well-preserved axonemes, the spokes themselves are not readily distinguishable from the matrix material, as shown in the past studies (4, 9, 15). In cross section, they are seen to be about 200 Å in width and, except for their heads, to show little structure (Fig. 1). However, Warner (15) clearly demonstrated in negatively stained preparations that they contain rodlike core structures 70–125 Å in thickness. He also indicated the probable existence of an additional component between pairs of spokes in sectioned material. In Fig. 2 *a* and *b*, a pair of faintly defined granular structures (small

arrows) is recognized between pairs of spoke heads. They may correspond to such a component.

Warner (15) suggested that the precise arrangement of paired radial links as well as their relation to the central sheath could have direct bearings on the sliding mechanism of peripheral doublets which was proposed by Satir (11, 12) for the cilial movement. It is possible that the fine structure of the spoke head reported here represents an important part of the mechanism.

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