THE ARRANGEMENT OF THE AXONEMAL MICROTUBULES AND LINKS OF *ECHINOSPHAERIUM NUCLEOFILUM*

W. F. HARRIS. From the Department of Chemical Engineering, Institute of Technology, University of Minnesota, Minneapolis, Minnesota 55455. Dr. Harris' present address is Departments of Chemical Engineering and Bioscience, University of the Witwatersrand, Johannesburg, South Africa

In their interesting paper Tilney and Byers (6) describe the arrangement of the microtubules constituting the axonemes of *Echinosphaerium nucleofilum*. The writer finds several points of theirs with which on geometrical grounds he cannot agree.

Fig. 1 shows an idealized cross-section of an axoneme with the positions of the microtubules shown by heavy dots. The microtubules are arranged in a double polygonal spiral with the array divided into 12 sectors by 12 clearly defined, approximately radial boundaries. The boundaries radiate from two central microtubules. There is evidence for the presence of macromolecular links connecting microtubules but, unfortunately, in the only published micrograph known to the writer which distinctly shows links (6), the arrangement is far from clear.

THE BOUNDARIES

Following MacDonald and Kitching (3), Tilney and Byers (6) arrange the links within a sector as shown in Sector 1 (S.1) of Fig. 1. The links are of two types, long links all parallel to one boundary (B.1) of S.1 and short links connecting adjacent

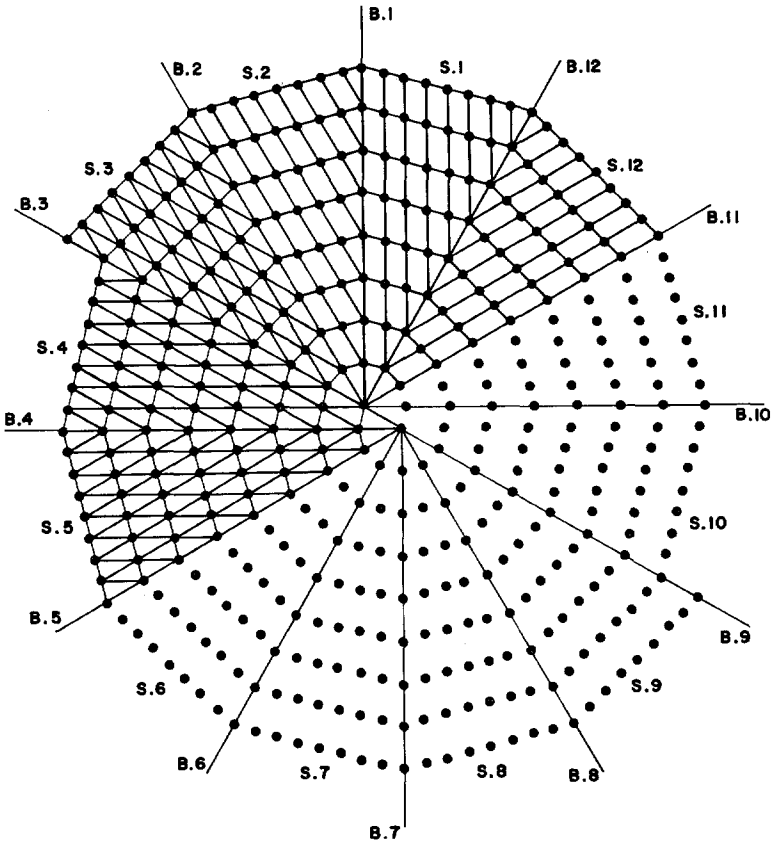

FIGURE 1 An idealized cross-sectional view of the axoneme of *Echinosphaerium nucleofilum*. The positions of the microtubules are shown by heavy dots. They are arranged in a double polygonal spiral starting from two central microtubules. The arrangement is divided into 12 30°-sectors, S.1, S.2, . . . , S.12, by 12 boundaries, B.1, B.2, . . . , B.12. Boundaries B.3 and B.9 are different from the rest: following one of the spirals outwards, one finds that each time one crosses B.3 or B.9 the number of tubules per sector increases by one. MacDonald and Kitching (3), Tilney (5), and Tilney and Byers (6) arrange links between microtubules as in S.1; there are short links connecting adjacent microtubules within a spiral and long links between spirals and parallel to one boundary (B.1). They also insert long links along the boundaries. As explained in the text, short links are not necessary; in each sector (long) links are arranged parallel to and along *both* boundaries of the sector as in sectors S.3, S.4, and S.5. All boundaries are symmetrical.

microtubules in a spiral. Passing anticlockwise to S.2 one finds long links at an angle of $\pi/6$ to those in S.1 and parallel to B.2. The long links are asymmetrically disposed on either side of B.1. The arrangement of Tilney and Byers (6) has all the boundaries like B.1.

In the electron micrographs there appears to be no evidence for this asymmetry: the micrographs show only symmetrically disposed microtubules. Clearly then, there is no apparent reason why the long links of S.1 should be parallel to B.1 and not B.12. The boundary asymmetry can be removed by arranging the links as on either side of B.12. To convert B.1 to a symmetrical boundary, one would then have to arrange the long links of S.2 parallel to B.1. Continuing thus around the axoneme, one would arrive at a structure with six boundaries like B.12 alternating with six like the new B.1. However, examination of the micrographs does not reveal any alternating difference between the boundaries that would support such a conclusion. All the boundaries appear to be of the same type,

and this leads one to the conclusion that the long links should be arranged as in S.3, S.4, and S.5. Thus there probably are two sets of long links, those in each set parallel to one boundary of the sector. (The members of the two sets do not differ in length.)

All the links in Fig. 1 are not necessarily in one plane. In fact, if the microtubules themselves are built up of subunits arranged on a simple helix with 12 subunits per turn (5), one might expect at most one link attached to each tubule in one plane. An electron micrograph, of course, might show more than that simply because the specimen has thickness.

IDEALIZED ARRANGEMENT

According to Tilney (5) the microtubules in the axoneme are "precisely spaced, a separation of 70 A between microtubules in each coil and a 300 A separation between adjacent coils." The spacings in the micrographs are, of course, not precisely of one value or the other. By making all long spacings identical and all short spacings identical, one obtains the idealized arrangement of Fig. 1. This is the arrangement implied by Tilney's statement quoted above.

The electron micrographs differ from Fig. 1 in two important ways. Near the center of the axoneme the boundaries are usually slightly curved in the same sense as the double spiral. Towards the periphery the rows of microtubules in the spiral are often not quite straight but bow in slightly towards the center. It is not difficult to see that both of these distortions could be artifacts due to shrinkage during preparation of the specimen for microscopy. The boundaries, as interpreted in B.3 and B.4, have a herringbone structure and are quite different from other radial lines. If any stresses were applied on the axoneme, say by isotropic shrinking of the embedding material, then one would expect the resulting radial strains to be different along boundaries than along other radial lines. Bowing in within the sectors is precisely what one might expect from such shrinkage. The spiral nature of the axoneme is likely to result in boundaries subjected not only to radial compressive stress but also to a bending stress.

LENGTHS OF AND ANGLES BETWEEN LINKS

If the links in each sector of the axoneme are arranged as in S.3, S.4, and S.5 of Fig. 1, it is obvious that all the short links can be removed without disrupting the structure. In other words, it appears that the short links postulated by MacDonald and Kitching (3) and discussed by Tilney (5) and Tilney and Byers (6) are unnecessary: the whole structure requires links of only *one* length.

Moreover, fixing the diameter of the microtubules and the length of the long link fixes the short spacing and hence the length of the short link (if it exists). If d_1 and d_2 represent the long and short spacings, respectively, then by simple geometry

$$d_2 = 2d_1 \sin \pi/12 = 0.5176\, d_1$$

If D is the external diameter of the microtubules and l_1 and l_2 are the lengths of the long and short links, respectively, then

$$l_2 = 0.5176\, l_1 - 0.4824\, D$$

Tilney and Byers (6) give l_1 = 300 A, l_2 = 70 A, and D = 220 A. These values are not compatible with the above equation. A suitable compromise which is compatible with the equation would be l_1 = 330 A, l_2 = 65 A, and D = 220 A. The corresponding spacings are 550 and 285 A. Examination of the micrographs shows that the difference between these figures and those of Tilney and Byers lies well within the range of experimental error.

Microtubules apparently are never found closer together than their short spacing (6). This might suggest that each microtubule is coated with a layer of material which does not stain or that the outer layer of the microtubule itself does not stain.

Tilney (5) and Tilney and Byers (6) imply that the angle between unstrained links on a microtubule is a multiple of $\pi/6$. However, their figures show a wide range of angles. Even in S.1 and S.2 of Fig. 1 with links arranged as they suggest, all the angles are not multiples of $\pi/6$; strictly they are all multiples of $\pi/12$. The angle between short links and long links is $5\pi/12$ or $7\pi/12$. If there are no short links, then it is true that the angle between links is a multiple of $\pi/6$ as is seen in S.3, S.4, and S.5 of Fig. 1. With such an arrangement every microtubule not on the periphery of the axoneme is linked to four neighbors, except the two central microtubules which are linked to eight neighbors. In the arrangement considered by MacDonald and Kitching (3), Tilney (5), and Tilney and Byers (6), micro-

tubules on the boundaries are linked to five others (two short links, three long links), microtubules within the sectors to four others (two short, two long), and each central microtubule to seven others (one short, six long). Fig. 17 of Tilney and Byers (6) is exceptional in that it shows one of the central microtubules linked to eight others (one short, seven long).

SPIRAL AND CONCENTRIC ARRANGEMENTS

By cutting Fig. 1 along B.3 and B.9 and making a displacement parallel to the cut and equal in magnitude to d_1, one can convert Fig. 1 into a concentric dodecagonal arrangement of microtubules. (One need not cut through the microtubules—one can cut the links on one side of B.3 and B.9 and rejoin them in the displaced position.) In such an arrangement each microtubule is again linked to four others, except for the central one which is linked to 12 others. (Even with the link arrangement discussed by Tilney and Byers (6), the same operation can be performed with a resulting central microtubule linked to 12 others.)

It is clear therefore that on the basis of the length of links alone there is no reason why the axoneme should not have a dodecagonal structure. If the axoneme was dodecagonal, then growth would not be steady; each time a dodecagon was completed, another would have to be nucleated. The rapid reformation of the axoneme after disruption probably demands a steady and organized growth mechanism which is provided by the spiral structure in the form of permanent steps. This is in close analogy with growth of a conventional crystal near the point of emergence of a screw dislocation (1) and with the growth of helical capsids of Tobacco Mosaic Virus (2, 8). Herein probably lies at least part of the reason for the spiral arrangement. Two microtubules each linking eight others may be more energetically favorable than one microtubule linked to 12 others; this could be a second contributing reason.

POSSIBLE ROLE OF DISLOCATIONS

The cutting operation described above is most suggestive. In crystal physics, displacements of this kind usually occur by the passage of dislocations (4). A dislocation with Burgers vector parallel to B.3 and B.9, and with magnitude d_1, moving on a glide plane parallel and adjacent to B.3 and B.9 would convert the spiral arrangement to the concentric arrangement. Passage through the axoneme in the opposite direction would bring it back to its original form.

If the dislocation moves only half way through the axonemal section, then the result would be a single polygonal spiral. A single spiral, however, is unlikely for any length along the axoneme because it would require an axial edge dislocation which is generally an unstable configuration (4).

It is interesting to note that dislocations might be involved in at least two other ways. In describing the transformation from normal microtubules to the enlarged variety, Tilney and Porter (7) and Tilney (5) explain that the globular subunits constituting the microtubule may slide past one another. This represents an example of simple topological contraction of cylindrical crystals which very likely occurs by the passage of dislocations (2). Tilney and Byers (6) suggest that "the tubules may slide past one another as in skeletal muscle." Nabarro (4) proposes that the sliding of filaments in muscle is due to dislocations. In fact, the relative sliding of any two structures which are joined together by regular bonds, links, or connectors probably occurs by the passage of dislocations or dislocation-like waves.

I would like to thank both Dr. R. P. Rhea of the Department of Genetics and Cell Biology, University of Minnesota, St. Paul, and Professor L. E. Scriven of the Department of Chemical Engineering, University of Minnesota, Minneapolis, for criticizing the manuscript and the latter for his continuing advice and encouragement.

This work is supported by the Air Force Office of Scientific Research under AFOSR Grant No. 219-67, L. E. Scriven, Principal Investigator.

Received for publication 5 December 1969, and in revised form 5 February 1970.

Note Added in Proof: Experimental verification of some of the above results appears in a new paper by L. E. Roth, D. J. Pihlaja, and Y. Shigenaka. 1970. *J. Ultrastruct. Res.* **30**:7. For the long and short spacings Roth and co-workers obtain 550 and 290 A, respectively. Their micrographs show little or no bowing in within the sectors, which supports the conclusion that the bowing in is an artifact in earlier micrographs. Roth et al. discard the description "double spiral" in favor of "paired series of half circles." While a "paired series of half *dodecagons*" is identifiable in the arrangement, "double polygonal spiral" is far more descriptive. In this sense the term "spiral" can raise no objection on geometrical grounds.

REFERENCES

1. FRANK, F. C. 1949. *Discussions Faraday Soc.* **5**:48.
2. HARRIS, W. F., and L. E. SCRIVEN. 1970. *J. Theor. Biol.* In press.
3. MACDONALD, A. C., and J. A. KITCHING. 1967. *Nature (London).* **215**:99.
4. NABARRO, F. R. N. 1967. Theory of Crystal Dislocations. Clarendon Press, Oxford.
5. TILNEY, L. G. 1968. *In* The Emergence of Order in Developing Systems. Developmental Biology Supplement. M. Locke, editor. Academic Press Inc., New York. **2**:63.
6. TILNEY, L. G., and B. BYERS. 1969. *J. Cell Biol.* **43**:148.
7. TILNEY, L. G., and K. R. PORTER. 1967. *J. Cell Biol.* **34**:327.
8. WATSON, J. D. 1954. *Biochim. Biophys. Acta.* **13**:10.