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ALTERNATING POSITIVE AND NEGATIVE TWIST OF POLYMERS IN AN INVERTEBRATE INTEGUMENT

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Abstract Here is presented the structure of the organic matrix of the protecting tube secreted by a deep-sea worm. This material shows an extremely original distribution of fibrils, reminiscent of the arrangement of polymers in cholesteric liquids, but it differs from true cholesterics by the twist handedness which is inverted after each 180° rotation. Right-handed layers alternate regularly with left-handed ones. The origin of this particular structure is discussed and, among plausible mechanisms, we consider a cellular control of fibril orientations and the motions of the worm within its tube.

INTRODUCTION

Many species of marine worms secrete protecting tubes, which are more or less mineralized. Specialized regions of the body are involved in the secretion of this shelter, regularly renewed at different steps of the growth.

We studied tubes from the species Alvinella pompejana (1) living in an extreme biotope, the deep-sea hydrothermal vents. These tubes appear to be made of a fibrous material consisting in extremely stable glycoproteins (2). Polysaccharides and proteins are covalently associated to form fibrils. There are also sulphur and mineral salts present in the tube wall. The whole structure is birefringent and large diameter fibrils are resolved in light microscopy. Semi-thin sections of these tubes show remarkable distributions of fibrils, with series of nested arcs, which recall certain biological analogues of liquid crystals and namely cholesteric systems. However, the structure considered here is very different (3-5) and our purpose is to present a three-dimensional reconstruction and to consider the possible morphogenesis of this

organization.

MATERIALS AND METHODS

Tubes of the annelid Alvinella pompejana were collected in the vicinity of the submarine hot springs by the submersible Cyana at 2600 m depth, in the Eastern Pacific Ocean, during the Biocyarise cruise in April 1984. They were preserved in ethanol or fixed in formol saline or simply rinsed in distilled water and air-dried. Young specimens were chosen for microscopical studies, since they are less mineralized, and this facilitates the preparation of sections. Small pieces of tubes were post-fixed in 1% osmium tetroxide and embedded in Durcupan. Semi-thin sections for light microscopy were stained with toluidine blue and ultrathin sections for electron microscopy were contrasted with uranyl acetate and lead citrate; they were examined using a Phillips TEM 201. Some samples, dehydrated in ethanol, were critical point dried and sputter coated with gold, to be examined using a Phillips SEM 505.

RESULTS

The tube of Alvinella is fibrous and concentrically multilayered. This is well observed in cross section. We call vertical a section plane lying normally to the tube surface and horizontal a section plane which is parallel to the tube surface or tangential. The other section planes are oblique. These definitions are local and are illustrated in Fig.1.

Sections which are exactly vertical (Fig.2) show that fibrils lie horizontally. Fibrils appear as separated dots, when seen in cross section, whereas they form short segments in oblique view and long segments in longitudinal view. All segments lie parallel to the tube surface and are therefore horizontal. Longitudinal fibrils alternate with fibrils seen in oblique view or in cross section. This indicates that fibrils follow different horizontal orientations.

Different patterns are observed in oblique sections and there is a

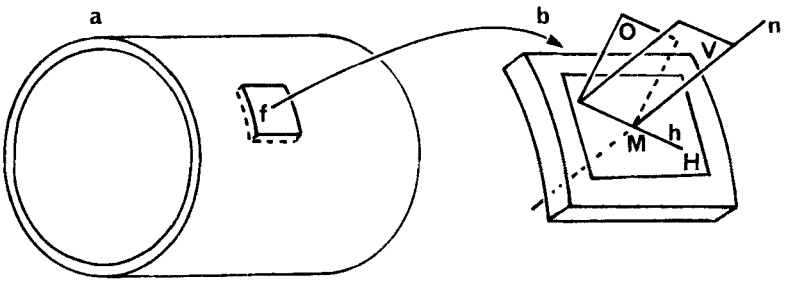

FIGURE 1 a. Part of worm tube, with a rectangular fragment (f) enlarged in b. The tangent plane H at point M of this fragment is said to be horizontal as well as the straight line h in plane H. Structures lying normally to H are said to be vertical and, for instance, the normal n to H in M, the plane V, defined by n and h, etc. The other orientations are said to be oblique, the plane (O), for instance, containing h and not n.

translational symmetry parallel to the tube surface (Fig.3 to Fig.5). Some of these patterns are schematized in Fig.6. The translation symmetry also exists in vertical sections described above. This indicates that fibrils lie parallel within any horizontal thin layer, but their orientation depends on the level considered in the tube wall. Let us call B the horizontal direction of this translation symmetry in the section plane; this direction B is that of bands of nested arcs, sinusoids or parallel sigmoids, presented in Fig.6. In the micrographs of oblique sections (Fig.3 to Fig.5), the B direction is also well specified and long horizontal segments represent fibrils in longitudinal or in nearly longitudinal view, whereas short segments correspond to fibrils in oblique section; they lie horizontally, as shown above, but they are not parallel to the B direction.

The structure of the integument is complicated by the presence of bacteria at certain levels, in the thickness of the tube (Fig.2), and by horizontal layers of the fibrous matrix which are either extremely dense, or extremely poor in fibrils (Fig.3). The origin of these differentiations is uncertain. Some dense layers of fibrils are probably the result of

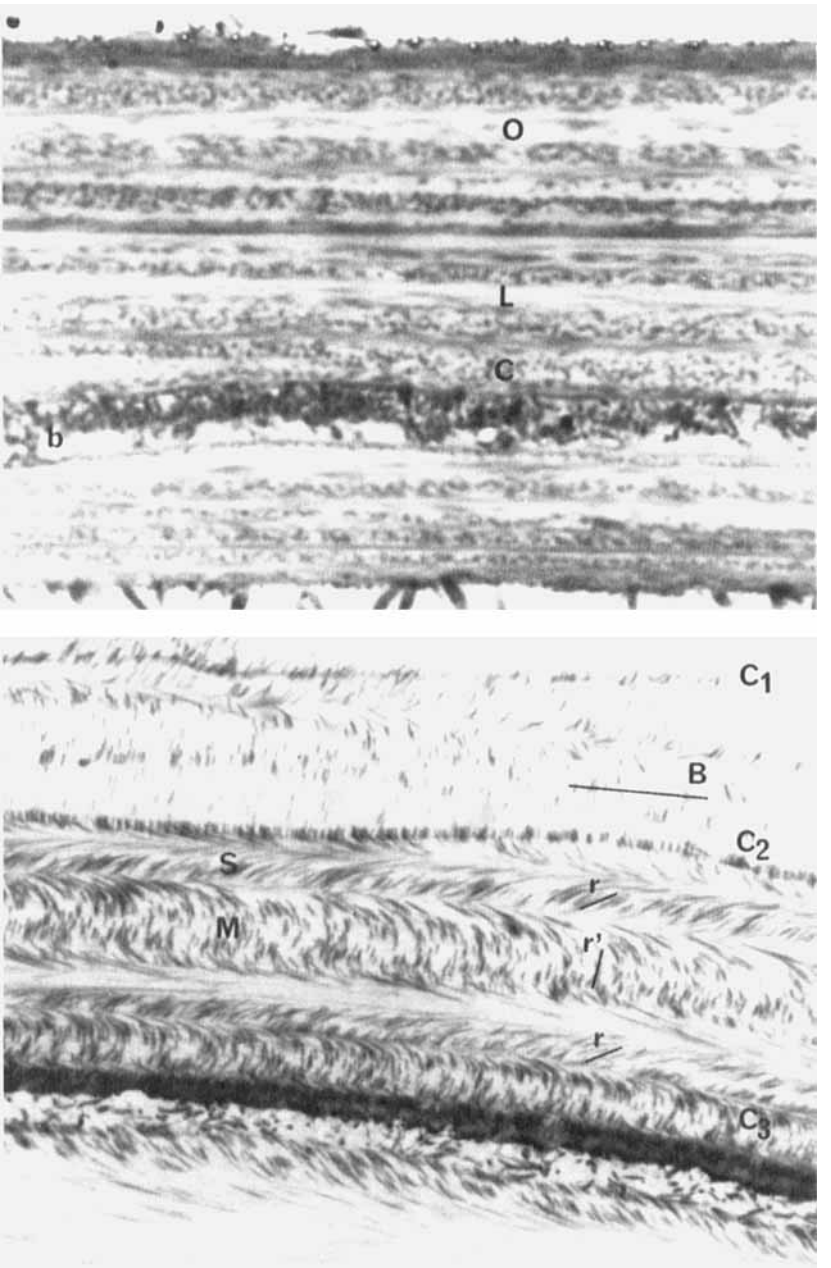

FIGURE 2 Vertical section of the tube. Fibrils lie parallel to the tube surface. There is a bacterial layer (b). Fibrils are in longitudinal view (L), in oblique view (O) or in cross-section (C).

FIGURE 3 Oblique section of the tube wall. Certain levels are particularly contrasted (C1,C2,C3). Sinusoid patterns (M) and sigmoid patterns (S) are observed. B,r,r': see the text and Fig.6.

microtomy artefacts (6,7).

Horizontal sections do not really exist, since there are no sufficiently flat pieces of tube. Some very limited areas in certain sections are horizontal but they are rare. However, it is not necessary to have exactly horizontal sections to observe the precise orientations of fibrils. Nearly horizontal sections are sufficient and show these orientations in the successive horizontal levels.

The fibril orientation presents discrete angular steps, varying from 20 to 60°, and series of four to six clockwise steps alternate with similar series of anticlockwise steps. Each series of rotations corresponds to a global rotation of 180° or slightly less. This is shown in Fig.7, a representation of a quasi-horizontal section of superimposed layers of parallel fibrils. Each band visible in the oblique section plane comes from a thin horizontal layer with an average orientation of fibrils, represented by a diameter in the corresponding dial (Fig.7). The diameters indicating the fibril orientation oscillate between two extreme orientations separated by an angle of nearly 180° or less. If this angle is really 180°, there is a unique orientation R of twist inversion. On the contrary, there are two slightly different orientations R and R', if this angle is less than 180°. Examples of this situation are presented in Fig.3 and Fig.5.

From the analysis of a lot of section planes, it can be shown that the fibril orientation R corresponding to the twist inversion is normal to the tube axis. When there are two different orientations R and R', the bissector of the angle R,R' (<90°), is normal to the long axis of the tube.

To summarize, the system presents a twist similar to that of cholesteric liquid crystals, but there are three differences:

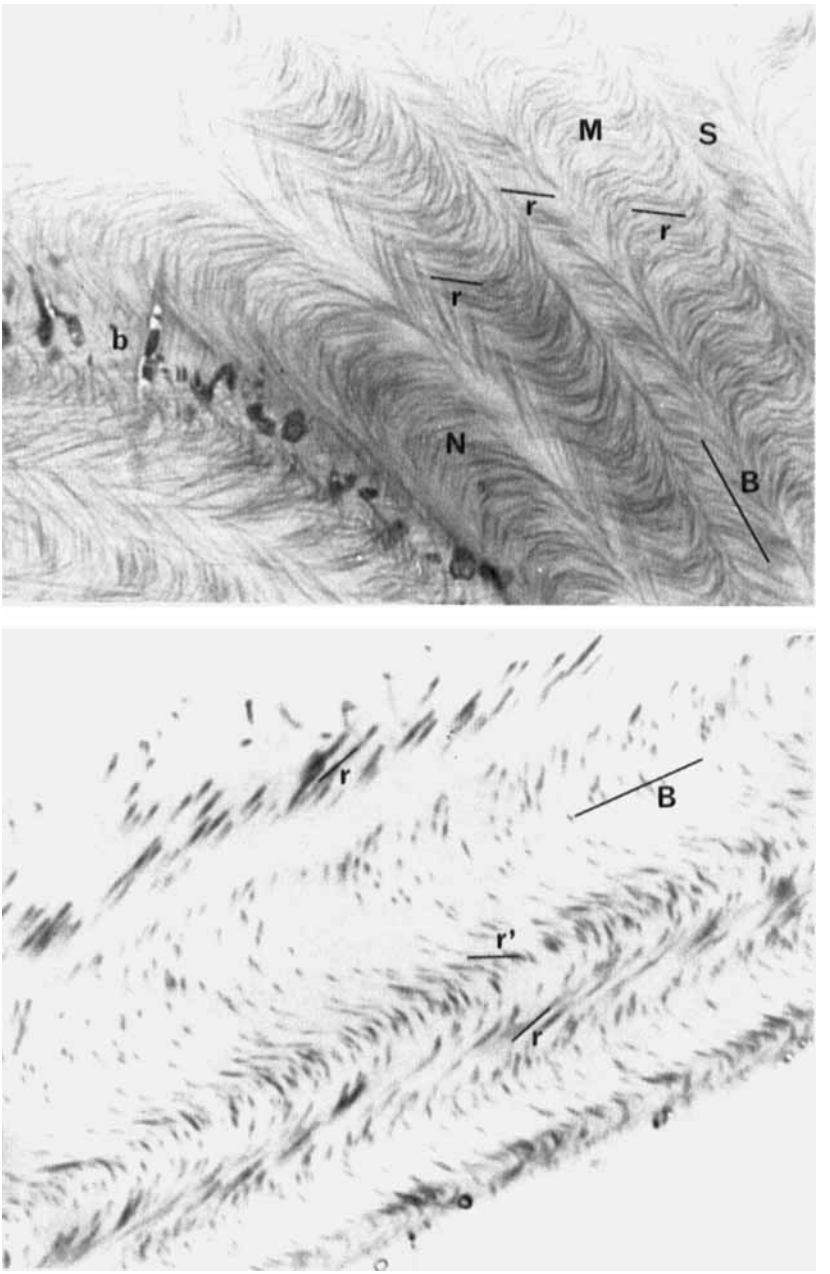

FIGURE 4 Quasi-horizontal section showing patterns similar to those of Fig.3. B: bacterial layer; M: sinusoid patterns; N series of nested arcs; S: sigmoid patterns. B,r,r': see the text and Fig.6.

FIGURE 5 Oblique section with sinusoid patterns. The arcs present different curvature radii, indicating different absolute values of the twist according to the handedness. B,r,r': see the text and Fig.6.

1. the twist is inverted after each rotation, by 180° or less
2. the twist is discrete
3. the tube wall is a system of stabilized polymers and is not liquid.

The oblique sections, lying far from horizontality, present patterns which are very similar to those observed in quasi-horizontal sections.

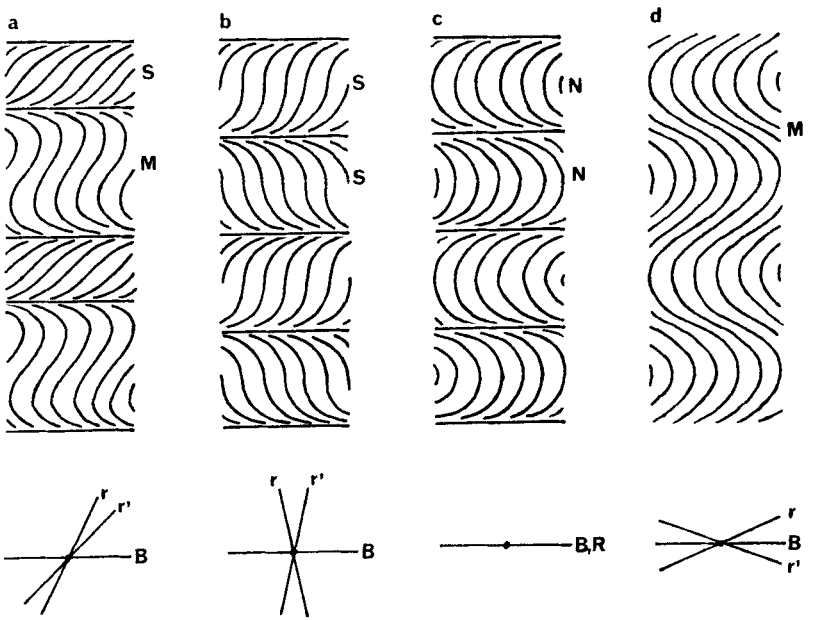

FIGURE 6 a-d: Theoretical examples of patterns arising fom different orientations of r and r' relative to B, corresponding to the translation symmetry within the section plane The R orientation in (c) corresponds to the case R = R', and R lying within the section plane. M: sinusoidal patterns; N: nested arcs; S: sigmoidal patterns.

Let us suppose that we are looking at a three-dimensional model of the structure, with an oblique section plane as shown in fig.7, but much steeper. The patterns observed in top view, normally to layers of fibrils, do not really differ from those seen in the oblique view, normally to the section plane. Nested arcs remain, sigmoids are kept, and the only differences correspond to slight curvature variations. The recognizable patterns do not change. The only variations of patterns come from the relative position of the B and R directions, and they are not related to the obliqueness angle. In micrographs, the B direction is observed directly, whereas R is known only from its projection r onto the section plane. The B and r orientations specify completely the type of pattern and are indicated in Figs. 2 to 7. There are cases with two orientations r and r' (Fig.3 and Fig.5).

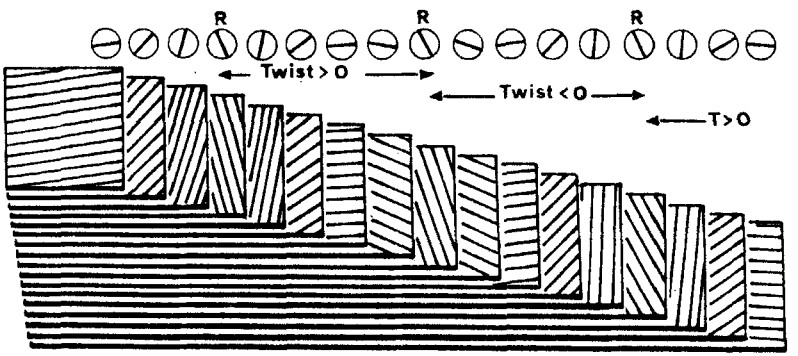

FIGURE 7 Schematic representation of an oblique section. The drawing is facilitated by a series of superimposed layers of fibrils. However, vertical sections indicate that such layers are not so well individualzed. A series of dials shows the progressive twist, its alternating handedness and the orientation R corresponding to the switch of the twist. In this case, R = R'.

X-ray diffraction studies of fragments of these tubes, with the X-ray beam oriented normally to the surface of the tube, show no preferential orientations of polymers, either at medium or at high diffraction angles

(2). This comes probably from the superimposition of all possible orientations of fibrils within the twisted layers.

In electron microscopy, ultrathin sections show patterns similar to those observed in light microscopy. There are large diameter fibrils, which are possibly polysaccharides (8) and in between thinner fibrils, with a lower order parameter, and densely aggregated around large fibrils (Fig.8).

DISCUSSION

The alternance of right-handed and left-handed twists, with equal rotations is very surprising. Several hypotheses can be formulated.

1. The polymer is normally nematic and two twisting factors of opposite handedness are secreted alternatively. However living systems synthesize only one sort of optical isomers. If the two factors correspond to different molecules, there must be a particular mechanism to get equal rotations on the right and on the left.
2. The polymer orientation is under control of cells which secrete the fibrils or depends on certain motions of the worm within its tube. Several works indicate the role of such motions, when new internal layers are secreted (9).

There are examples which show a left-handed cholesteric twist, with distortions due to the presence of cells, or muscular attachments (4). The orientation of fibrils is the result of a cholesteric self-assembly and of orientations due to cellular structures. In the case of the tubes of marine worms, the body rotation and certain cellular controls are probably essential in the orientation of glycoprotein fibrils, since such twist reversals are unknown in liquid crystals.

Sections of tube walls show well defined patterns due to the varying orientation of fibrils, with inverted series of nested arcs or parallel sigmoids (Fig.6). Some of these patterns with a translation symmetry are

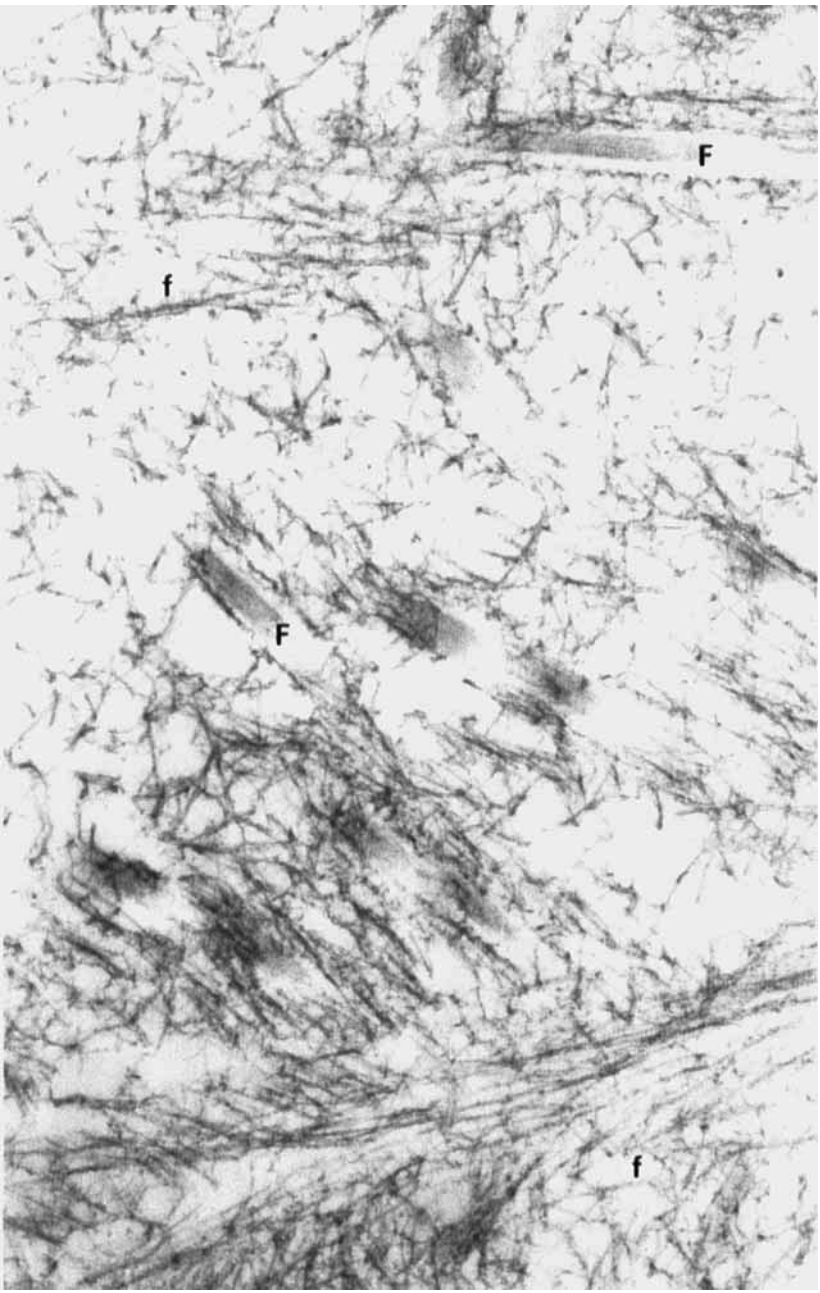

FIGURE 8 Large diameter fibrils (F) and thin diameter fibrils (f) observed in the electron microscope.Thin fibrils are less ordered thand thick ones and form cylindrical aggregates around large fibrils.

reminiscent of what is expected in section from a particular nematic structure, with constant twist, constant bend and zero splay. This geometry is also that of force lines representing the molecular alignments in chiral smectics C (10). This was our first interpretation, in the absence of vertical sections, when we had only oblique views, which are the most numerous. However the analysis of different section planes and the absence of arcs in vertical sections lead us to the only possible model, the twist inversion, which was initially difficult to accept, after many works on cholesteric analogues.

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