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<td>HIRAKAWA, Yasuhiko</td>
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A SEM Observation of Microtubules in Xylem Cells
Forming Secondary Walls of Trees*

By
Yasuhiko HIRAKAWA**

走査電子顕微鏡による樹木の二次壁形成中の
木部細胞における微小管の観察*

平川泰彦**

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Introduction

Since the discovery of microtubules in plant cells by Ledbetter and PORTER,1 many instances of agreement in orientation between microtubules and microfibrils have been shown2-8. It has been agreed by many workers, therefore, that microtubules adjacent to the cell wall are involved in orienting the microfibrils as they are deposited9-11. On the contrary, as the examples of inconsistency of orientation between them12 and that microtubules are sometimes absent even when a cell is laying down exceedingly well-ordered microfibrils have been reported,13 the other functions of microtubules have also been suggested by some workers14-17. It has been generally noted that microtubules have various functions in animal cells, so they probably function in a variety of ways in plant cells. In the present work, therefore, the SEM observation was made in order to clear how microtubules were involved in the structural features of secondary wall of tree xylem cells rather than only the microtubule function itself.

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** Laboratory of Wood Physics, Faculty of Agriculture, Hokkaido University.
** 北海道大学農学部林学科 木材理学講座
In the elucidation of correlation between microtubules and microfibrils from a morphological point of view, the orientation and localization of them must be clear in cells having more complicated wall structures during all cell differentiations. In the past the transmission electron microscopy have been a standard technique for this purpose, however, there seems to be a limit in ability of clarifying the orientation of filamentous structures such as microtubules and microfibrils by the ultrathin sectioning method as already pointed out\textsuperscript{18,19}. From this point of view, a SEM study possesses an advantage of being able to make a not only of orientation of microtubules but also of localization of them and further cytoplasm/wall features in comparison with the examination by sections, but the value of this technique for such studies is not generally appreciated. Thus, the author has shown in the earlier papers the three-dimensional configuration of cell organelles and their spatial arrangement in xylem cells can be clear by SEM\textsuperscript{20} and further the relationship between microtubules and depositing microfibrils both in non pitted\textsuperscript{20} and pit border regions\textsuperscript{20} of differentiating tracheids can be clearly elucidated by it.

It is well known that the secondary wall sturucture of xylem cells of tree is fairly complicated, various between types of cells\textsuperscript{28} and variable in vessel elements between tree species\textsuperscript{50}. As already indicated,\textsuperscript{24-26} in the case of vessels, the structure of the secondary wall is often complicated by the presence of spiral thickenings which consist of bundles of microfibrils and are sometimes running in various directions even in a cell. It seems to be of interest, therefore, that specimens of this kind are observed by the SEM for the knowledge of the correlation between microtubules and depositing microfibrils. This relationship has been examined in tracheids and fibers of trees,\textsuperscript{4,7,18,14,19,21,22} however, little is known concerning the orientation of microtubulrs in differentiating vessel elements. As the more recent work\textsuperscript{40} by the author could clear the microfibrillar orientation and the wall thickening process in vessel elements of some species, the present work was made in order to clear the correlation between microtubules and microfibrils in these cells using SEM. Also, as the fine details of correlation between microtubules and depositing microfibrils still remain to be resolved in cells forming secondary walls of trees, the critical morphological observation of the microtubules was made by means of a new type of SEM equipped with a field emission gun and the morphological association between microtubules and depositing microfibrils in other cells forming secondary walls of trees was examined by the replica method.

For the observation of microtubules in differentiating vessel elements, 3 species diffuse porous woods, Azukinashi, Hōnoki and Asada, having three distinct types of spiral thickenings and a species, Doroyanagi, not having it were examined. The structures of vessel secondary walls of these species have already been published by OHTANI and ISHIDA\textsuperscript{25} and by the author and ISHIDA\textsuperscript{40}.

This paper is a portion of a thesis for the degree of Doctor of Agriculture from Hokkaido University, December 1982.
Materials and Methods

Materials for this study were obtained from Todomatsu (Abies sachelinensis MAST.), Karamatsu (Larix kaempferi CARR.), Azukinashi (Sorbus alnifolia C. KOCH), Hônoki (Magnolia oovvata THUNBERG), Asada (Ostrya japonica SARG.) and Doroyanagi (Populus maximowizii HENRY), in Tomakomai Experiment Forest, Hokkaido University.

Small wood blocks containing xylem, cambial zone and phloem (about 5 x 5 x 5 mm) were prefixed in 3% glutaraldehyde in 0.05 M sodium cacodilate buffer for 2-6 hrs at room temperature (about 18°C) and then 5 hrs at 4°C. Following prefixation, materials were washed in ten changes of 0.15 M buffer for 2 hrs. The materials were then post fixed in 2% osmium tetroxide in 0.05 M buffer for 2-3 hrs at room temperature or 4°C.

After the fixation, for the SEM observation, they were dehydrated in graded series acetone, the next dried by the critical point drying method, and then cut radially by the razor blade in order to disclose the lumen surface of the cell. Specimens were coated by Pt using the vacuum evaporated coating method, or shadowed by Pt and then coated Au by the ion sputtering coating method. In the specimen for the field emmision SEM observation, the coating thickness of Pt seemed to be under 50 Å.

With regard to the method for the replica technique, the author described in the earlier paper²².

Observations were made with JSM-F 15 (Field Emission SEM), JSM-35 CFII and JEM-6AS.

Results

In the SEM observation of cortical microtubules, the near inner surface of the plasmamembrane must be scrutinized. Generally differentiating fusiform cells were highly vacuolated and there were little cytoplasmic matrixes in active season, therefore, specimens of this kind were more suitable for the SEM study of cortical microtubules.

When viewed with the SEM, microtubules were found as slender and cylindrical structures on the plasmamembrane, the diameter of which was 250-300 Å and nearly uniform in a longitudinal direction (Photo 1). The terminations of microtubules were dispersed randomly in a cell. A great majority of them were from 3 to 5 microns in length, but they were sometimes reached above 7 microns as shown in Photo 2. The space between microtubules was various from 100 Å (center to center above 350 Å) to 1 microns in non pitted regions of differentiating tracheids (Photo 2) and fibers, but this was rather constant than in the same region of vessel elements forming S 3 or spiral thickenings (in the case of Azukinashi including S 2) in which microtubules were specifically localized as will be described later. It was ascertained that the passage of microtubules through the plasmamembrane was not present and other cellular organelles except for the plasmamembr-
brane which was jointed to microtubules by bridges were not associated with microtubules. These features above mentioned were generally common to three types of cells, as tracheids, fibers and vessel elements forming secondary walls.

With regard to the orientation and the localization of microtubules in differentiating tracheids of a conifer (Todomatsu), the author already reported that the regular changing pattern of orientation accompanying with a cell differentiation was in agreement between microtubules and depositing microfibrils in both non pitted and pit border regions, and such features were also as same in Karamatsu. In

![Fig. 1. The regular changing pattern of microtubules and microfibrils accompanying with the cell differentiation and the relationship between the two in differentiating tracheids.](image1)

Mt: Microtubule  
Mf: Microfibril

![Fig. 2. The relationship between microtubules and microfibrils in the pit border regions between tracheids forming the S2 layer. The pit chamber side is shown on left hand and the lumenside on right hand.](image2)

Mt: Microtubule  
Mf: Microfibril
this paper, therefore, the author describes about only the relationship between them in a cell. Photo 3 shows microtubules (left hand) and depositing microfibrils (right hand) in a cell which commenced to form a lamella of S3 on the S23 layer of Todomatsu. The orientation of microfibrils of the innermost lamella is in a flat-helix and in agreement with that of microtubules. Additionally, the number of microtubules appears to be nearly as same as that of depositing microfibrils (in a flat-helix) in this photo and further it seems to be found that 1–2 microfibrils per a microtubule are equivalently formed under each microtubules. The relationship between microtubules and microfibrils as they are deposited is drawn in Figs 1 and 2, a non pitted region and a pit border region, respectively, of tracheids undergoing the secondary wall formation.

The secondary wall in the vessel elements of four species consisted roughly of S–Z–S microfibrillar inclination for S1, S2 and S3, respectively, but the S3 layer was often modified even in the non pitted regions. In the case of Azukinashi vessel elements, spiral thickenings in Z-helix were already formed even in the late stage of S2 formation. The structures of S1 and S2 are generally similar to that of tracheids in three species vessels except for S2 of Azukinashi and both the orientation and the localization of microtubules in the vessel elements forming S1 and S2 were also as same (Fig. 1), but there were many variations of microtubules orientation and localization in vessel elements forming S3 and the spiral thickenings. The results of observation of microtubules in the non pitted region of a vessel element being modified are described as under.

In mature vessel elements of Azukinashi, there are large spiral thickenings of Z-helix and small ones of S-helix. The former is the ridges of the part of S2 and the latter is the part of S3 or the distinct ridges itself on S3. Photos 4A and 4B show the microtubules and the depositing microfibrils, respectively, in the cell forming the spiral thickenings of S2 in Z-helix. In these photos it is clear that microtubules are closely arranged on two ridges and oriented in parallel to the spiral thickening direction of Z-helix. On the contrary, there are no microtubules in the area of the groove between two Z-helix spiral thickenings. At the late stage of the S3 formation, both Z-helix microfibrils and S or flat-helix ones are simultaneously deposited (Photo 5A). In the cell at a same differentiating stage, microtubules were also oriented in Z-helix on the spiral thickening of same-helix and the orientation of some of them changed into S-helix (Photo 5B arrow). In other regions where the microfibrils of S3 are deposited uniformly and there are no spiral thickenings, microtubules were also present as in the same fashion of the tracheids forming S3 layer. A relationship between microtubules and depositing microfibrils in a cell forming S3 is drawn in Fig. 3.

In the case of Hônoki, it is characteristic that the thinner parts of S3 layer like as ribbons are present. These structures are formed by microfibrils which are deposited in the fashion of ribbons or bands when the S3 layer commenced to form (Photo 6A). The appearance on the plasmamembrane is shown in Photo 6B at the same differentiating stage. In these photos, it is revealed that the region of
microtubules aggregation and other region of smooth surface of plasmamembrane (Photo 6 B arrows) are alternately present in the fashion of ribbons as same as of S 3 microfibrils. And also the appearance of tapering ends of such smooth surface (near upper arrow) was similar to that of grooves of S 3. This relationship between is drawn in Fig. 4.

In the case of Asada, the inclination of spiral thickenings is irregular even in a cell and further the additional layer, the microfibrillar orientation of which is distinct from that of S 3 and variable between vessel elements and even in a cell, is often present. Therefore it is difficult to examine the correlation of orientation between microtubules and microfibrils in a cell, but it was same features as other species that microtubules were closely present in the region where the wall thickening was undertaken at a later differentiating stage of S 3 (Fig. 5). The instance that the orientation of microtubules is also variable even in a cell is shown in Photo 7. They are axially oriented in the non pitted region, on the other hand, some of them are directed in flat-helix and crossed the region between pits.

In vessel elements of Doroyanagi, the secondary wall nearly consists of typical three layers and S 3 is little modified in the non pitted region. As microtubules
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near the pits have been examined in differentiating tracheids (Fig. 2), the near pits in differentiating vessel elements was examined in this species because which had comparatively simple wall structure. The features of microfibrillar deposition near the pitted region between a vessel element and a ray parenchyma cell were characteristic in a cell forming S3 layer. As shown in Photo 8 A, the microfibrils are deposited in flat-helix at this stage, therefore, the wall thickening of narrow regions between pits (arrow) is little undertaken. In this region the orientation of microtubules was also linear not through the narrow region between pits (Photo 8 B).

The microtubules in differentiating fibers were oriented and localized in all stages of the secondary wall formation as same as in tracheids of conifers (Fig. 1). The examples of the orientation of microtubules in fibers forming S1 and S2 of Hōnoki is shown in Photos 9 A and 9 B, respectively.

In the past, it has been noted that the Golgi bodies are associated with the cell wall formation and also some Golgi vesicles are associated with the microtubules giving the appearance that they have trapped or not the Golgi vesicles. From this point of view, the SEM observation of the Golgi bodies was also made in order to clear the relationship between it and microtubules. The three dimensional structure of the Golgi body is shown in Photo 10 (A, B and C) at three differentiating stages of secondary wall formation in tracheids. When viewed with the SEM, the features of Golgi bodies were various in a cell, the one has complex feature which is composed of tubules and vesicles (10A), and the other has narrow flattened cisternae (10B) or wide one (10C) in a central region and tubular proliferations extended from it. It is of interest in these photos that the Golgi bodies appear to be in contact with tubular membranes which are supposed to be smooth endoplasmic reticula (s-ER) and further these membranous components may be finally fused into the plasmamembrane (Photo 10, arrow). The Golgi vesicles were frequently seen at the edges of cisternae, but it was not ascertained that they were released toward the plasmamembrane or not although various vesicles were often observed near the plasmamembrane. As the connection between dictyosomes and the endoplasmic reticula was shown earlier, similar results that vesicles were in contact with the tubular membranes or plasmamembrane were observed. The

Fig. 5. A correlation between microtubules and microfibrils in a vessel element forming the additional layer and spiral thickenings on the S3 layer. A dotted line shows the region where the microfibrillar deposition is undergoing.
Mt: Microtubule
Mf: Microfibril
relationship between vesicles derived from dictyosomes and microtubules has been indicated by some workers, however, the specific localization of vesicles near the microtubules or not was not recognized and there were no relationship between microtubules and Golgi bodies and between microtubules and vesicles near the plasmamembrane.

**Discussion and Conclusion**

There has a little been reports concerning the microtubules in plant cells using SEM, but little SEM study was undertaken concerning the fine detail of the general morphology of them, so it was firstly examined. It was revealed in this study that the specimen preparations for SEM provided little artifacts for the features of microtubules. For instance, the microtubule diameter observed in the current SEM study was 250-300 Å (Photo 1), therefore, it is clear that artificial increasing of it by the metal coating was restrained under about 50 Å comparing with the average value being about 240 Å gained by the TEM study. And also, even the cylindrical feature of microtubules could be detected in the SEM images, so the current SEM preparations might produce nearly the best overall morphological preservation. With regard to the fine structure of microtubules the TEM study has an advantage from the point of view of a instrument resolution, however, the SEM study had rather an advantage in the morphological observation, because it permitted a clear view of not only the orientation and localization of microtubules but also their terminations, length and so on.

In Photos 1 and 2, the terminations of microtubules were clearly shown and their length could be seen in the latter. A great majority of the ends of microtubules as detected by SEM seem to be true terminations, because there are no possible continued microtubules near ones having the terminations. This proposal is also fortified by the result of that there were no microtubules under about 3 microns in length. If they are frequently separated artificially by the SEM preparations, the extreme short microtubules must be seen. As microtubules seem to leave their structure and feature intact in spite of damage done to specimens by drying, cutting coating and so on, it is suggested that cortical microtubules are rather stiff and rigid structures. And also such good preservation of microtubules in SEM specimens seems to be owing to their specific structures that microtubules were joined to the plasmamembrane by the bridges. They were generally 3-5 microns in length, therefore, it is clear that they are not forming complete rings and continuous spirals in a cell. This result is similar to that of other plant cells as indicated by the serial sections, but it is remarkable that a few long microtubules were also surely present in many cells and they were sometimes reached above 10 microns in length. These findings suggest that the length of microtubules is possible to be variable in many types of cells and even in a cell, but it is of interest that they are always lengthened above 3 microns in tree cells.

More recently, the microtubule organizing center is assumed to be present in cell corners. In this study microtubules which were oriented in various directions
were examined in many cells and the many terminations of which were observed, but there were no possible points which could be suggested to be organizing centers of microtubules. Microtubules were keeping parallelism even in cell corners (Photo 9 A), and even where the orientation of them was changed in a cell and they were closely neared, their terminations were always differently present. Therefore, there was no direct evidence in the case of xylem cells of trees that the two microtubules were directed from an organizing center in cell corners and the mechanism of controlling the microtubule orientation is present in the same region.

With regard to the relationship between microtubules and microfibrils in differentiating tracheids, the detail of it in a cell forming the S 3 layer was only examined (Photo 3). The significant point in this observation is that the correlation between microtubules and depositing microfibrils was indicated in a cell having a crisscrossed structure, i.e. it can be compared between only the newly-deposited microfibrils recognizing the forming lamella and cortical microtubules. If the specimen of this kind is observed by the ultrathin sectioning method, the only few microfibrils of the inner most lamella as shown in Photo 3 seems to be not detected. This evidence may be suggested that some instances of inconsistency of orientation between the two as reported previously are misconceived. From this observation, it is clear that the orientation of microtubules was consistent with that of microfibrils of the innermost lamella. Additionally, in a cell just after the lamella formation commenced, the agreement not only of orientation but also of the number of the two is clear.

In vessel elements forming the S 1 and the S 2 layer except for Azukinashi (S 2) and in fibers forming S 1, S 2 and S 3 layer (Photo 9), the changing pattern of microtubule orientation accompanying with the cell differentiation was similar to that of tracheids forming same layers as illustrated in Fig. 1, except that the angle of them is more or less different between tree species.

A correlation between microtubules and depositing microfibrils was illustrated in Figs. 3–5, and in Photos 4–8, in vessel elements at a later differentiating stage. It is remarkable in these cells being modified wall that (1) microtubules are definitely present only in the area where the wall thickening is undertaken and (2) in spite of the presence of some groups of the aggregation of microtubules and difference in orientation in a cell, the orientation between microtubules and underlying microfibrils is always consistent. The agreement of orientation between the two was not directly demonstrated in vessel elements in comparison with as shown in tracheids (Photo 3), but this can be concluded from the observations on the process of microfibrillar deposition and secondary wall thickening as the author reported previously. For instance, the direction of spiral thickenings generally reflects the microfibrillar orientation in this structure, therefore, it can be suggested that if the orientation of microtubules is in agreement with the direction of spiral thickenings, both the orientations between microtubules and underlying microfibrils are same.

From the study of microtubule orientation and localization by SEM in three types of cells of six species, the general assumption is noted just below, in all
stages of the secondary wall formation, the orientation of microtubules is always in agreement with that of underlying microfibrils (Photos 3–9, Figs. 1–5) and microtubules are definitely present in the region where the wall thickening is undertaken in both non pitted (Photos 4–6, Figs. 3–5) and near the pitted regions (Photo 8, Fig. 2). And further, their orientation and localization are possible to be variable accompanying with the cell differentiation (Fig. 1) and with the difference in the wall structure (Fig. 2). From view of these considerations, it is suggested that microtubules are involved in orientation of microfibrils and in the place of microfibrillar deposition.

Many workers have concluded from the results of the agreement of orientation between microtubules and microfibrils that microtubules are involved directly or indirectly in orienting microfibrils and are in close relation to the developing thickenings. In contrast, the disagreement of directions between the two and the disappearance of microtubules in some types of cells have been reported, so other functions are concluded. If the function of microtubules is cytoskeletal, to govern cytoplasmic streaming or channeling into the wall substances, the evidence that the orientation of them is regularly changed accompanying with the cell differentiation and is variable each regions in relation to the wall structure (Photos 5 A, 5 B and 7) can not be interpreted. The absence of microtubules during the main phase of secondary wall formations of trees was reported, however, they were always found in all cells undergoing the same wall formation in all cases examined. And also, it was suggested that material apparently excuded from the cut ends of microtubules might be consist of wall matrix substances, however, as pointed out by the other worker, it was ascertained that no evidence for the presence of microtubules outside the plasmamembrane was found. Some workers reported that microtubules were seen associated with all vesicles derived from the dictyosomes which appeared to be moving towards the wall. As the features of Golgi bodies will be discussed later, dictyosomes vesicles were closely continuous with the smooth ER through tubules of cisternae and finally attached to the plasmamembrane (Photos 10 A and 10 C), therefore, there seems to be doubtful with regard to the correlation between microtubules and intracellular vesicles derived from dictyosomes. And also, the morphological association between them was not found in tree cells.

Although the mechanism that microtubules transfer their influence to the plasmamembrane is not well known, in my opinion, the moving particle theory introduced by Chafe et al. seems to possess certain advantages. By this assumption that the one of function of microtubules is directional control of enzyme complex which are especially concerned with the formation of the organized microfibrils, the morphological association between the two as shown in this study can be reasonably interpreted. For instance, it can be concluded that the relationship between the two in a cell as shown in Photo 3 indicates the features just after the re-orientation of microtubules prior to the deposition of a new wall lamella although a few microfibrils have already been deposited in a cell. The reason why
instances of the difference in orientation between the two as pointed out CHAFE and WARDROP, and FUJITA and HARADA was not found seems to be that microfibrils are rapidly deposited just after the re-orientation of microtubules. And further, this assumption seems to be fortified by the evidence that 1-2 microfibrils per a microtubule appear to be deposited under each ones (Photo 3).

It has been reported that there were at least two sets of microtubule orientation or it was irregular, but these were observed in cells forming primary walls in many cases. The organization and the way of cell wall formation are different between the primary wall and the secondary wall, hence the two should not be simply compared. Similar observations were made on the cells forming the primary wall of trees by the author (unpublished data), so the microtubules at the primary wall formation should be further examined in future.

By the colchicine treatment it is well known that the orientation of depositing microfibrils is disturbed, but the deposition is keeping. In this study, it was apparent that there were no microtubules where the secondary wall was not thickened, i.e. pit membrane, the flattened regions in a cell forming only the spiral thickenings and the area between pits as shown in Photos 8A and 8B. From the result by the colchicine treatment it is concluded that microtubules are not always involved in the microfibrillar deposition, however, the current investigation seems to indicate that microtubules are also involved in determining the place of microfibrillar deposition.

With regard to the Golgi bodies, the SEM micrographs of Photos 10A, B and C bring into clear view not only of 3 dimensional structures of them but also of the association between tubules attached to a cisterna and the smooth ER. From the result of the continuity between Golgi bodies and the smooth ER, it is suggested that this structure is involved in the transport of wall precursors component, because as reported previously a great majority of organelles composed of single membrane, i.e. smooth ER, rough ER, vacuolar membrane and plasmamembrane, consist of continuous structures. It is generally interpreted that the transport of wall substances is undergoing by the release of mature vesicles from the dictyosomes, however, the continuity between the Golgi bodies and the plasmamembrane through the smooth ER might indicate that the transport is undertaken by this structure. Large vesicles attached to the tubules of cisternae may only reflect the strong activity of Golgi bodies. Therefore there might be no relationship between microtubules and all vesicles near the plasmamembrane as indicated in this study. Although the morphology of such organelles is shown by SEM the observation of this kind provide immediately little evidence that they are involved in the cell wall formation or not. There is the possibility, however, the a better understanding of cell wall formation is acquired, because when the SEM autoradiography can be applied to the study of this kind in future, the morphological study would provide an important information.

From the above results and discussion it is apparent that there is the morphological association between cortical microtubules and depositing microfibrils in
cells forming secondary wall of six tree species. Such results have already been reported by many workers in many cells, however, the most significant point from this study is that the fine details of the relationship between the two were clearly resolved in cells forming complicated walls using SEM. It is difficult to detect the orientation of microtubules which are directed variously in a cell by the ultrathin sectionning method, and in the observation of the microtubule length and localization the SEM study has also advantage. Furthermore there is the possibility that the SEM study of cellular organelles brings into clear view the function of the Golgi bodies and the other organelles in the recent future.

Acknowledgment

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References


要約

树木の二次壁形成中の木部細胞に存在する微小管 (Microtubule) の配向および分布様式を、走査電子顕微鏡 (SEM) により観察し、二次壁構造 (ミクロフィプリル配向) との関連からその機能を考察した。

針葉樹二種 (トドマツ、カラマツ) および広葉樹四種 (アズキナシ、ホオノキ、アサダ、ドロノキ) の三種の細胞 (仮道管、道管要素、繊維状仮道管) を対象として観察した結果を要約すれば以下のとおりである。

細胞分化にともなう微小管の配向の変動様式および分布様態は、一細胞内では non pitted region と pit border で異なり、細胞の種類ごとに、また道管要素では樹種間で異なる。しかし、以下の点で堆積時のミクロフィプリルと形態的相互関係を有する。

1. 両者の配向が細胞分化にともなって規則的に変動し、かつその変動パターンが一致する。
2. 原形質膜を介して接する両者の配向が一致する。
3. 微小管の存在は、二次壁厚が継続されている領域 (ミクロフィプリル堆積が行われている領域) に限定される。
4. ラメラ形成の開始時には、両者の個体数がほぼ対応する。

以上の事実から、微小管は、堆積時のミクロフィプリル配向の決定のみならず、ミクロフィプリル堆積の場の決定に関与しているものと結論した。
Explanation of Photographs

Note: Longitudinal direction of wood is vertical in all photographs

Photo 1 Cortical microtubules in a differentiating tracheid forming the S2 layer of Todomatsu. They are about 250 Å in diameter and their terminations (arrows) are shown. (A field emission SEM micrograph)

Photo 2 The length of cortical microtubules in a differentiating tracheid forming the S2 layer of Todomatsu. They are often reached 7 µm in length. (A field emission SEM micrograph)

Photo 3 A photograph by the replica method showing microtubules and depositing microfibrils in a differentiating tracheid forming the S3 layer of Todomatsu. The orientation of microtubules (left hand) is in agreement with that of only innermost microfibrils in flat-helix.

Photo 4 Differentiating vessel elements forming the S2 layer of Azukinashi
A Orientation and localization of microtubules. They are oriented in Z-helix and localized closely on the spiral thickening.
B A lumen surface of a vessel element forming the cell wall. Microfibrils are oriented in Z-helix and two ridges of spiral thickenings are directed as same.

Photo 5 Vessel elements at a later differentiating stage of Azukinashi
A A lumen surface of a vessel element forming the cell wall. The bundles of microfibrils are forming two S-helix spiral thickenings (arrows).
B Orientation and localization of microtubules. They are definitely localized and the orientation of some of them is changed from Z-helix to S-helix (arrow).

Photo 6 Differentiating vessel elements forming the S3 layer of Hōnoki
A A lumen surface of a vessel element forming the cell wall. Two bundles of microfibrils like as a ribbon are depositing (arrows).
B Orientation and localization of microtubules. They are oriented in flat-helix, but they are not present in the area as shown by arrows.

Photo 7 Orientation of microtubules in a differentiating vessel element forming an additional layer on the S3 of Asada. They are oriented in steep-S-helix in the non pitted region, but flat-helix and sinuous in near pitted regions.

Photo 8 Near pitted region (a vessel element and a ray parenchyma cell) of differentiating vessel elements forming the S3 layer of Doroyanagi.
A A lumen surface of a vessel element forming the cell wall (replica method). Microfibrillar deposition is not undergoing in the narrow region (arrow) between pits.
B Orientation and localization of microtubules. They are oriented in flat-helix and not localized in the narrow region between pits.

Photo 9 The orientation of microtubules in differentiating fiber tracheids of Hōnoki.
A The orientation of microtubules in a cell forming the S1 layer. They are oriented in flat-helix. Membranes (left hand) are endoplasmic reticula (ER).
B The orientation of microtubules in a cell forming the S2 layer. They are oriented in Z-helix.
Golgi bodies in differentiating tracheids of Karamatsu.

A. Golgi bodies showing several tubules and vesicles in a cell forming the S1 layer. The ER is connected with the plasmamembrane (arrow).

B. The typical Golgi body in a cell forming the S2 layer.

C. A widely flattened cisterna and vesicles of the Golgi body in a cell forming the S23 layer. Vesicles are formed at the edges of the cisterna (black arrow). Tubules are attached to the smooth ER (white arrows).