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WHAT ARE TRANSCELLULAR STRANDS?

By

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With 15 Figures in the Text

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A. Introduction

In two recent publications, THAINE (1961, 1962) advanced the hypothesis that transcellular protoplasmic streaming is the major cause of translocation in plants. The streaming, as revealed by particle movement, is supposed to occur throught transcellular strands 1 to 7 μ in diameter extending from cell to cell across the walls. In the supporting photomicrographs, the entities labelled transcellular strands are longitudinally oriented lines, unnaturally straight for cytoplasmic structures. They remind one of diffraction lines, from cell walls which are not in the focal plane, commonly seen in thick free-hand sections. The identification of sieve plates is another doubtful element in these papers.

We have examined the structures described by THAINE as transcellular strands and are reporting our observations in the present paper.

B. Material and methods

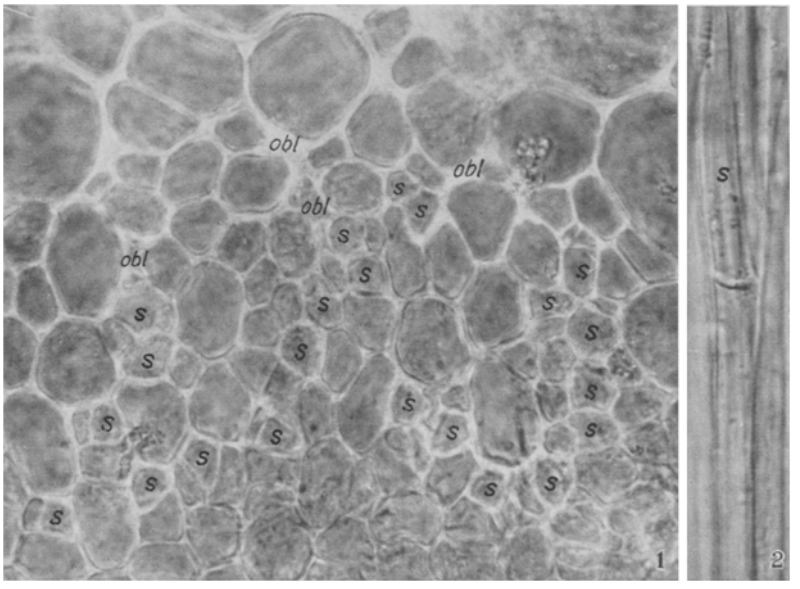
The material consisted of petioles of *Primula obconica* HANCE, one of the species used by THAINE (1961). Free-hand sections were observed either fresh in tap water or after treatments designed to kill or completely destroy the cytoplasm. Some sections were frozen, thawed out, and then placed in about 0.1% papain solution in distilled water overnight. Others were treated for short times in 5% NaOH or 95% alcohol. The photographs included in the paper are those made with ordinary transmitted light, but the observations were supplemented by phase microscopy.

C. Observations

The phloem in the single vascular bundle of the *Primula obconica* petiole resembles that of many other dicotyledon petioles. Narrow sieve elements associated with still narrower companion cells are distributed in groups among somewhat wider parenchyma cells (Fig. 1). Still wider parenchyma cells appear on the periphery of the phloem. This is the protophloem in which the sieve elements are crushed and obliterated (*obl* in Fig. 1) during extension growth of the petiole. The large parenchyma cells of the protophloem elongate more extensively than the deeper lying cells. The sieve elements have transverse or slightly

inclined simple sieve plates (Fig. 2). Callose has been identified in these by means of anilin-blue staining and fluorescence microscopy.

In fresh longitudinal sections mounted in tap water, cytoplasmic streaming may be observed without any difficulty in the wider and narrower parenchyma cells. The movement of small plastids readily reveals the streaming even at ×500 magnification, especially in the long



Figs. 1 and 2. Fresh sections in tap water. Magnification: ×1600

Fig. 1. Transection of phloem with most sieve elements indicated by letters *s*. At *obl* more or less crushed sieve elements being obliterated

Fig. 2. Longitudinal section with part of sieve tube at *s*. Sieve plate, thickened by callose, in median position. Starch granules near the sieve plate would stain reddish brown if treated with iodine

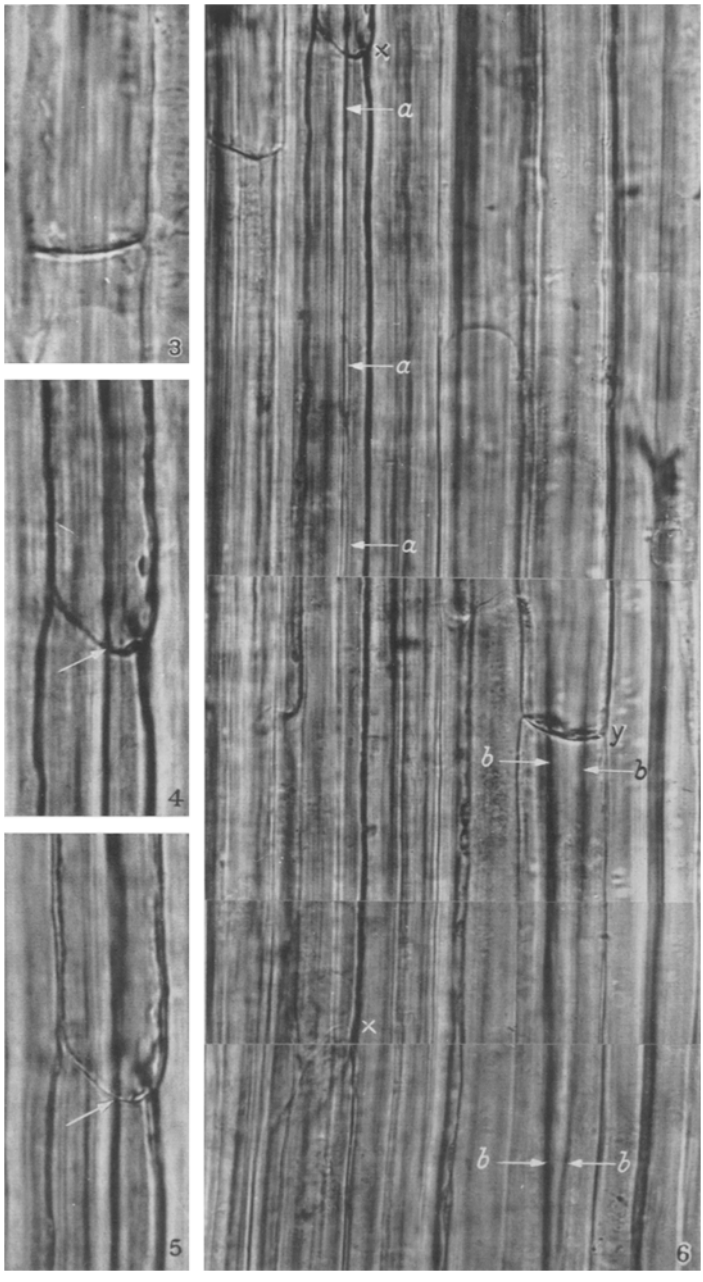
and wide cells at the periphery of the bundle. The cells are highly vacuolated so that the movement is restricted to the narrow layer of cytoplasm along the walls. As usual, the movement is reversed when it reaches the end wall; and streaming cytoplasm remains confined to a single cell. No streaming occurs in the mature sieve elements, and their starch grains (Fig. 2) remain stationary.

Fig. 3. Longitudinal diffraction lines cross the transverse end wall in median position

Fig. 4. At arrow, a wall in focus crosses the end wall in median position

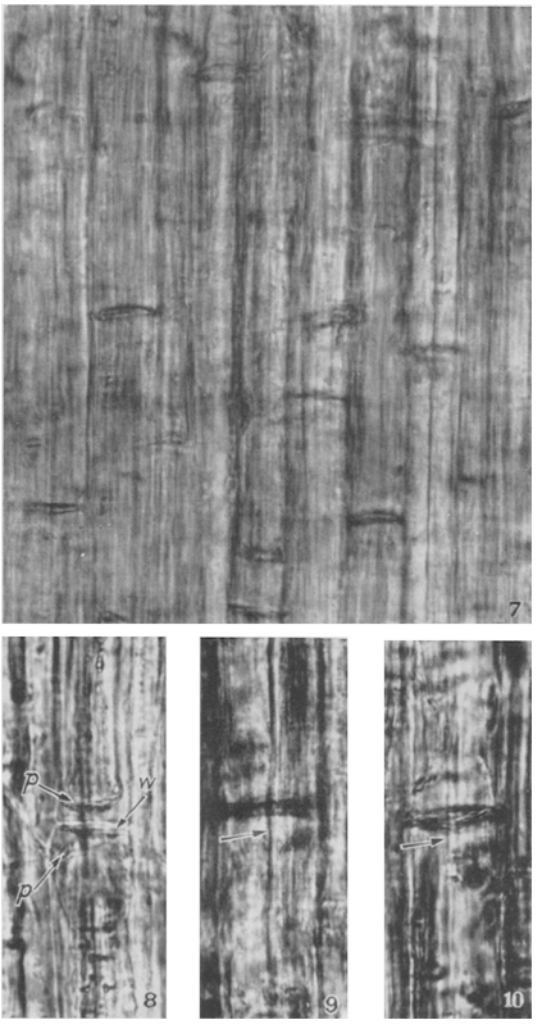
Fig. 5. At arrow, the same wall as in Fig. 4 is out of focus and appears to pass through the end wall in median position

Fig. 6. The wall labelled *a* is in focus above (solid line), out of focus below (broken into several diffraction lines). The walls marked *b* appear to pass through the transverse wall marked *y*. The ends of one long cell are seen at *x*. Numerous longitudinal diffraction lines throughout section



. 3—6. Longitudinal sections of parenchyma from outer part of phloem. Fresh sections in tap water. Magnifications: Figs. 3—5, ×1750; Fig. 6, ×1000

Figure captions on the bottom of the opposite page



Figs. 7—10. Phloem parenchyma of petiole. Sections frozen and treated with papain overnight, mounted in distilled water. Magnification: ×1250

Fig. 7. Numerous fine longitudinal diffraction lines throughout section

Fig. 8. The protoplasts *p* have withdrawn from the transverse wall at *w*. Longitudinal diffraction lines cross the protoplasts and the transverse wall

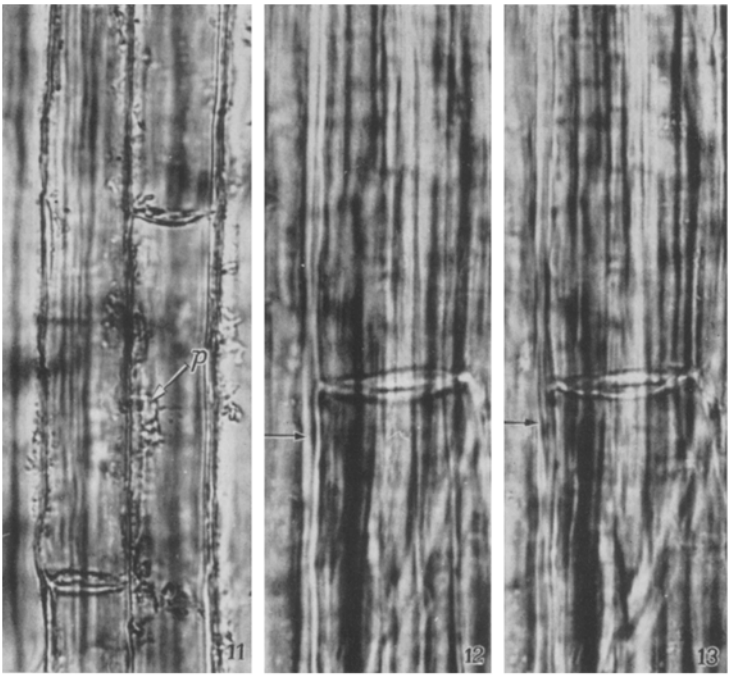
Fig. 9. At arrow, a wall in focus crosses the end wall in median position

Fig. 10. At arrow, the same wall as marked with arrow in Fig. 9 is out of focus and appears as two diffraction lines

Fig. 11. Remnants of digested protoplast at *p*. To the left, longitudinal diffraction lines appear to pass through the transverse wall below

Fig. 12. At arrow, longitudinal wall in focus. Numerous longitudinal diffraction lines appear to pass through the transverse wall in median position

Fig. 13. At arrow, the same longitudinal wall as in Fig. 12 is out of focus and is resolved into numerous lines



Figs. 11—13. Longitudinal sections of phloem parenchyma. Fig. 11, section frozen and treated with papain overnight. Figs. 12 and 13, section treated with 5 % NaOH for $^1/_2$ hr. Magnification: ×1250

Figs. 14 and 15. Longitudinal sections of phloem parenchyma killed in 95 % alcohol. Magnification: ×1250

Fig. 14. Wide parenchyma cells from outer part of phloem with contracted protoplast at *p*, and inclined end wall above. Diffraction lines cross the end wall

Fig. 15. Narrow parenchyma cells from inner part of phloem with a dense pattern of diffraction lines

A slight shadowing by means of the condenser diaphragm reveals numerous diffraction lines oriented parallel with the longitudinal walls of a given cell (Fig. 3). By changing the focus, it is possible to relate these lines to cell walls . In Fig. 6, for example, the arrows labelled *a* indicate a wall which appears as a single line where it is in focus (above) and as several lines where it is out of focus (below). Changing the focus creates various artifacts also in the spatial relations of different walls. In Fig. 4, for example, the crossing of walls at arrow indicates the junction of four cells. In Fig. 5 the longitudinal wall between two of these cells is out of focus and appears to be passing through the end wall between the other two cells. In Fig. 6 two longitudinal walls marked *b* appear to be passing through the end wall at *y*.

To preclude possible arguments that not all of the straight parallel lines result from diffraction but that some are indeed cytoplasmic strands, we present in Fig. 7—15 photomicrographs of sections in which no living cytoplasm was present.

Figs. 7—11 are from sections treated with papain. Fig. 7 shows a profusion of lines in a section treated with papain. The lines are more numerous and finer than in Fig. 6 because the section in Fig. 7 was cut somewhat deeper and included more of the narrow-celled phloem than that in Fig. 6 (see Fig. 1). In Fig. 8 the protoplasts (*p*) of the two superimposed cells are seen withdrawn from the transverse end wall, but the parallel diffraction lines are not disturbed. Figs. 9 and 10 give another example of the effect of focusing upon the appearance of the wall: one line in Fig. 9, two lines in Fig. 10. Fig. 11 is particularly instructive. It shows a combination of fragments of disrupted protoplasts with sharp straight diffraction lines.

The cells in Figs. 12 and 13, taken from a section treated with NaOH, show the usual diffraction lines. They are somewhat less numerous than, for example, in Fig. 7: the large cells in Figs. 12 and 13 were derived from the outer phloem where the number of walls in a unit of volume is smaller than deeper in the tissue. The arrows at the left in these two figures once again show that change in focus may break up the image of a single wall (Fig. 12) into numerous diffraction lines (Fig. 13).

Treatment with alcohol (Figs. 14 and 15) also does not affect the diffraction lines, which in Fig. 14 appear in combination with a severely contracted protoplast. The diffraction lines in Fig. 15 closely resemble those labelled transcellular strands in Plate I, Fig. 3, of THAINE's 1962 article.

D. Discussion

Obviously, we think THAINE's transcellular strands to be artifacts. His motion picture, a copy of which we have seen, probably displays cytoplasmic streaming in parenchyma cells which are longer than the

motion-picture frame. The picture, therefore, rarely shows the reversal of the streaming at the end of a cell and gives the impression that the sources and ends of bidirectional flow are some distance from the observer.

As was shown in Figs. 4, 5, 6 and 14, it is possible to focus in such a way that cross walls of some cells seem to be traversed by the longitudinal walls of other cells. When streaming occurs along these walls it appears to pass through the transverse walls.

The "sieve plates" of THAINE'S (1961,1962) photographs and in his film resemble end walls of parenchyma cells rather than those of sieve elements. A convincing identification of sieve elements in *Primula obconica* should be based upon 1. small diameter of cells, 2. presence of companion cells, 3. presence of starch grains which stain reddish brown with iodine, 4. thickness and evidence of pores in sieve plate, and 5. presence of callose on sieve plate.

Summary

The transcellular strands described by THAINE (1961, 1962) and interpreted by him as providing the structural basis for a transcellular cytoplasmic streaming are merely lines caused by diffraction of light from walls out of focus. These lines are as clearly visible in dead cells as in living.

Zusammenfassung

Die transcellulären Stränge, die THAINE (1961, 1962) beschreibt und als Wege einer transcellularen cytoplasmatischen Bewegung betrachtet, sind lediglich Linien, die durch Lichtdiffraktion von Zellwänden verursacht werden. Diese Linien können in lebenden sowie toten Zellen beobachtet werden.

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