Morphogenesis in *Micrasterias*

II. Patterns of morphogenesis

By T. C. LACALLI

*From the Department of Biology, McGill University*

**SUMMARY**

The final form of the polar lobe and lateral wings of developing semicells of *M. rotata* results from combined action of three growth processes: tip growth, branching and lobe broadening. Tip growth unaccompanied by branching or broadening occurs during normal development in *M. radiata*, but is observed only under abnormal conditions (e.g. raised temperature) in *M. rotata*. When branching and broadening do occur, they occur together and for this reason may be causally related. Autoradiograms demonstrate that specific patterns of cell wall incorporation can be associated with each of the three processes in *M. rotata*. Autoradiographic patterns found in the polar lobe differ from those found in wings. The growing polar lobe also responds to laser irradiation differently from the wings; lasings occasionally cause duplication of the polar lobe.

**INTRODUCTION**

In the first article of this series (Lacalli, 1975), lobes of the wings in developing semicells of the desmid *Micrasterias rotata* were shown to grow by tip growth in a fashion similar to that by which fungal hyphae are known to elongate. It can hardly be said that the finished wing (Fig. 1A) resembles a hypha, however, for in the process of development *Micrasterias* wing lobes branch repeatedly. But neither do the completed wings of *M. rotata* much resemble branched hyphae. Clearly several growth processes are involved in semicell development besides tip growth itself. This article deals with observations on these additional processes and their respective contributions to final semicell form. Attempts made to separate the processes either experimentally or by observing such separation in the normal morphogenesis of species other than *M. rotata* are included.

**MATERIALS AND METHODS**

The same *M. rotata* cultures were used as in previous work (Lacalli, 1975); *M. radiata* was isolated in March 1971 from Loon Lake near Haney, B.C., by Marion McCauley. Both species were cultured in the MS medium of Waris under conditions described previously for *M. rotata*. Information on the participation of specific regions of cell wall in morphogenesis was obtained by

---

1 Author's address: Bellairs Research Institute, St James, Barbados.
irradiating cells at various stages of development with a laser microbeam after treating them in solutions of alcian blue. To reveal patterns of cell wall incorporation, autoradiograms were prepared of primary cell wall ghosts from cells left for varying lengths of time during development in solutions of methyl-[H]-methionine and C-1-[H] glucose in culture medium. Detailed methods for both laser irradiation and autoradiography have been previously reported (Lacalli, 1975).

RESULTS

The pattern of semicell growth in _M. rotata_ could be altered by introducing various chemicals and enzymes into the culture medium or by adjusting its osmotic pressure during semicell development. _M. rotata_ cells were plasmolysed by concentrations of sucrose greater than 0.2 M, and growth was arrested without visible plasmolysis at concentrations from 0.1 to 0.2 M. At sucrose concentrations between 0.06 and 0.1 M, some but not all lobes were arrested or retarded in their growth, giving the semicell a generally abnormal or asymmetrical appearance. When plasmolysed cells were returned to culture medium, subsequent growth produced some monstrous forms (see, for example, figures in Kiermayer, 1964; Lacalli, 1973), but of such variety that it was difficult to generalize as to the type of abnormality to be considered characteristic. The enzymes pectinase and pectin methyl esterase prevented semicells from developing fully. The enzymes arrested or retarded lobe growth, and frequently lobes appeared swollen or more rounded than was normal. Swelling of lobe tips was also observed in cells treated with UV light, though the tendency for lobes to elongate without branching was most characteristic of this treatment. This observation agrees with other published reports on UV irradiation (Kallio, 1963; Selman, 1966).

It may be said of all the above experimental treatments, with the possible
exception of UV irradiation, that the more monstrous semicells produced appeared monstrous only because some individual lobes were stunted or the shapes of their tips altered while other lobes were unaffected. In no case was the pattern of lobe growth fundamentally altered. A somewhat more striking alteration in lobe form could be produced, however, by allowing semicells to develop at increased temperatures. Up to 35 °C, the pattern of semicell growth remained unaffected, and the rate of growth increased as one might have expected. At 38 °C semicell growth was arrested, but between 36 and 37.5 °C semicell lobes elongated substantially and abnormal forms were produced (Fig. 2). As with UV treatment, elongating lobes were unbranched.

The form of semicell wings in *M. radiata* can be more easily imagined to be a consequence of tip growth combined with lobe branching than can wing form in *M. rotata*. Wings of *M. radiata* resemble branched hyphae in that individual lobes are relatively narrow and of uniform diameter throughout their length, while branches are fixed at large angles relative to one another (Fig. 1 B). Analysis of developmental patterns in *M. radiata* revealed additional important differences between this species and *M. rotata*. Development in *M. radiata* could be divided into three phases (Fig. 3). During the first phase (stages 1–4) the semicell increased in size throughout while its two wings and polar lobe became established. Development was very similar to that occurring between stages 1 and 7...
Fig. 3. Changes in the perimeter of a growing *M. radiata* semicell stages 1–7 (A) and stages 7–9 (B). Consecutively numbered stages are separated from one another by 20 min intervals. The inset emphasizes the change in shape of the lower wing lobe (shaded) between stage 7 and stage 9.

Fig. 4. Changes in the perimeter of a growing *M. rotata* semicell. Consecutively numbered stages are separated from one another by 20 min intervals, but only odd-numbered stages are drawn here. Profiles are shown at 10 min intervals for two notches (circled). Note the continuing change in the position of these notches.

in *M. rotata*. Growth from stage 5 to 7 in *M. radiata* had no parallel in *M. rotata*, however. During this second phase, *M. radiata* lobes elongated without further branching and without any change having appeared in the positions of the lobe notches. At stage 7 growth changed again; lobes branched, and a broadening occurred across the body of the major wing lobes that was greatest at their bases. During this final phase, the positions of notches were substantially shifted. In *M. radiata* therefore the major period of lobe elongation is temporally separated from periods during which other growth processes, notably lobe branching and broadening, make their contribution to semicell form. These processes are not so separated in *M. rotata*; lobe notches change position continuously except for the most well-established notches, and branching is more frequent and regular than in *M. radiata* (Fig. 4). If the same three processes – tip growth, branching
Morphogenesis in Micrasterias. II

Fig. 5. The veining pattern of methionine label for primary cell wall ghosts from cells of different stages exposed to methyl-[\(^{3}\)H] methionine for 40 min. (A) The labeling pattern for a stage-12 semicell. (B) The labeling pattern for a stage-10 semicell. (C) A semidiagrammatic representation of regions of dense and uniform tip label (solid) and of veined label (shaded) for a stage-14 semicell.

and lobe broadening—operate in both species, their effects are in *M. rotata* more subtly blended.

Autoradiograms of primary cell wall ghosts from *M. rotata* cells revealed that wall incorporation was concentrated at the tips of lobes throughout development (Lacalli, 1975). In addition, autoradiograms revealed a secondary pattern on the surface of semicells somewhat resembling the veins in a leaf (Fig. 5; see also fig. 13F in Lacalli, 1975). This 'veining' was secondary in the sense that the density of vein labeling in any particular semicell was 50–100 times less per unit area than the tip label in the same cell. In late stage semicells (stages 11–13) with very densely labeled tips, the veined pattern was therefore evident and its dimensions could easily be traced (Fig. 5A, C). At earlier stages (stages 6–10) with less densely labeled tips, the veined pattern was less conspicuous but nevertheless present (Fig. 5B). Veined patterns were present in walls whether labeled with glucose or methionine. As in the case of tip label, veining in methionine-labeled walls was dense and sharply bounded while glucose label was more diffuse. Unlike the tip case, the area labeled in veining appeared to be about the same for methionine as for glucose. And the labeled regions did not increase in size with increasing duration of exposure to the labeled compounds, but instead became only more densely labeled. Fig. 5C was compiled from several such densely labeled walls and shows diagrammatically the regions of well defined and uniformly dense labeling. In general, veins of label followed the midline of wing lobes, branching wherever the lobes branched and becoming much wider at the branch points themselves. There were specific regions of the
wall which never labeled: a band of unlabeled wall always encircled the polar lobe at its base, and areas about the lobe notches were also unlabeled.

Veining of the polar lobe differed from that of the wing lobes in a curious way. The midline of the polar lobe was unlabeled, and instead veins ran along either side of the midline and united at the base of the polar lobe. The response of the polar lobe to laser irradiation was also curious. The entire polar lobe could be eliminated by lasing the central point of contact between daughter semicells at various developmental stages (Lacalli, 1975, figs. 3, 7). Lasings of wing lobes produced similar results; lobes were eliminated only if the lobe tip was irradiated. Occasionally, however, lasings intended to prevent polar lobe formation instead caused two perfectly formed polar lobes to be produced
Morphogenesis in *Micrasterias*. II

![Diagram of branching in cylindrical lobes](fig8.png)

Fig. 8. The geometry of branching in cylindrical lobes. (A) A single lobe. The wall contained in the growth hemisphere shown (solid) will, when fully extended, form a cylinder of wall as shown. If the single initial growth zone (indicated by an ×) duplicates, or additional zones are formed, all structure resulting from these new growth activities will finally lie above the dotted line. (B–D) Three variations showing the abutment of two branches atop the fully extended form of the lobe shown in (A). The lobes are shown in section, but it is assumed that a smooth continuous surface joins the original cylinder with its branches. Shaded areas are flat projections of regions on the three-dimensional surface of the final structure which are not accounted for in the surfaces of the cylinders themselves. (B) The case of two branch lobes, each half the diameter of the original. The additional surface required to complete this structure can be seen in the end-on view. (C) The case of two larger branch lobes which can only fit atop the original lobe without deformation if their bases are tilted. (D) The case of two large branch lobes as in (C) with additional broadening of the original lobe in association with branch formation. Note that this broadening decreases the tilt of the branch lobes relative to the axis of the original lobe.

(Fig. 6). This phenomenon had no parallel in lasings of wing lobes. In addition, lasing of the polar lobe tip could at late stages prevent further growth at the center of the lobe without preventing growth and elaboration of one or both lateral prongs (Fig. 7).

**DISCUSSION**

Where uniform cylinders of cell wall are found in the *Micrasterias* semicell, the process of tip growth may be considered to have been responsible for their formation (Lacalli, 1975). Branches, however, indicate that growth of a fundamentally different type has been imposed because constraints of geometry prevent branches from forming unless additional insertions of cell wall are allowed. This can best be shown by drawing attention to the ways in which variously sized cylinders can be fitted together in branched configuration so as to form structures without surface discontinuity (Fig. 8). For this figure it is assumed that the growth site or singularity responsible for the single lobe shown in Fig. 8A is to become responsible for two such lobes and so initiate a branch. Cell wall which was produced prior to this change will be extended by
subsequent growth to form a complete cylinder (shown extending to a dotted line in Fig. 8). All wall which forms after the change and arises from the new growth sites will, in the final structure, be found atop this original cylinder. Whether the new lobes are relatively small (as in Fig. 8B) or similar in diameter to the original lobe (Fig. 8C), two such lobes with flat bases cannot be set atop the single original cylinder without spaces being left. The respective surfaces do not join smoothly unless the circular bases of the new lobes are considerably deformed. If deformation is not to be allowed, it must be assumed that new surface is formed or existing surface is expanded to close up the spaces left. Since cell wall is formed continuously without leaving surface gaps later to be filled in, it is perhaps more accurate to say that the process of tip branching must be accompanied by cell wall growth elsewhere than at the lobe tip that compensates geometrically for the change from one growth site to two.

It is possible to envisage several mechanisms by which compensation might be avoided. The possibility of deformation or compression of the wall has already been mentioned. However, evidence consistent with the supposition that regions of wall must be filled in or expanded by additional synthetic activities is available for *M. rotata* from autoradiographic patterns in cell wall ghosts. For in the veined pattern, extensive labeling occurs at the points at which lobes have branched and at which the above analysis demands insertion of additional wall. Here the veins are always substantially wider than elsewhere, though they are not exactly triangular as the analysis predicts. The fact that labeling is observed in these regions long after branches have formed does not deny a special role for these regions in branch formation. It is probable that the regions labeled do so because they are qualitatively different from the surrounding wall which has been shown to be a product of tip growth. And it is reasonable to suppose that this difference can be traced to peculiarities of the original formation of these regions at the time of branching; presumably these regions were then the sites of some growth-related activity differing from tip growth.

Autoradiography also allows a narrow but continuous band of wall incorporation to be traced along the midline of semicell lobes to connect the wider regions. This pattern corresponds to that which would be expected if a general broadening of *M. rotata* wing lobes occurred by the insertion of new wall material along the midline of lobes (Fig. 8D). Such broadening of lobes is more apparent in *M. radiata* from stages 7-9 of semicell development, but probably also occurs to a lesser extent throughout development in *M. rotata*. Lobe tips progressively narrow during growth in both species to become finely pointed at late stages. In the finished semicell, therefore, major semicell lobes differ in size from their smaller branches only partly because they have been broadened during their later development. The difference exists to some extent because the size of the growth region at the lobe tip is progressively reduced during this same period.

It is interesting to note that branching and the broadening of lobes are associated with one another in the sense that they occur together in the cases so far
observed. This is true during normal growth of *M. rotata* and from stages 7 to 9 in *M. radiata*. From stages 5 to 7 in *M. radiata* and in *M. rotata* grown at 37 °C, tip growth may be observed in isolation unaccompanied by either broadening or branching. It is possible that the latter two processes are therefore causally related; either one depends on the other or both are responses to an additional, perhaps underlying, process. It could be supposed, for example, that the growth sites (singularities) responsible for tip growth were discrete structures capable of duplication, and that such an event would cause stresses within existing wall that would allow insertion of additional wall material in a veined pattern. There appears to be nothing inherently reproductive about singularities, however, since in *M. rotata* the polar lobe can elongate for a considerable length of time without branching, as can wing lobes if the temperature is elevated. It is therefore more reasonable to suppose that branching is stimulated by an activity external to the singularity itself. Suitable external influences could be supplied by the insertion of extra wall at points near the tips but disposed non-uniformly around their circumference. Or stresses on the wall as a whole could cause patterns of strain to arise within its structure stimulating as a response either singularity duplication, insertion of extra wall or both together. The evidence presented does not allow any of the alternatives to be especially favored.

The polar lobe also demonstrates tip growth, but leaves behind a cylinder of wall that is qualitatively different from that produced by branching wing lobes. There is no median vein of label in the polar lobe; instead two veins are present running along either side of the midline. Polar lobe growth also differs from wing growth in its response to laser irradiation. At early stages, the entire polar lobe can be eliminated by lasing at its tip. At later stages, lasing at the tip prevents continued growth of the lobe's central portion, but does not prevent formation of the lateral prongs. Therefore, despite the similarity of form between these prongs and the terminal dentation of wings, the prongs do not appear to arise as direct branches of the polar lobe at its tip in the same way that wing dentation is formed by branching of the more proximal wing lobes. On the other hand, the prongs usually branch at least once themselves. This branching process may be analogous to that found in wing lobes.

In conclusion, morphogenesis of the semicell wings in *Micrasterias* is due to three processes: tip growth, branching and lobe broadening, and to their assembly into an elegantly interlocking whole. Specific patterns of cell wall incorporation can be associated with each process. Under certain circumstances tip growth may be observed in isolation from branching and broadening, but the latter two have not as yet been observed in isolation from one another. An exact reconstruction of the functional or causal interactions among the three processes, though it would considerably improve our understanding of morphogenesis in *Micrasterias* and in other plant cells, is unfortunately not possible on the basis of the evidence presented.
This work was supported by the National Research Council of Canada and was carried out at the University of British Columbia.

REFERENCES


(Received 17 May 1974, revised 28 August 1974)