**INTRODUCTION**

Cell shape formation in desmids, and particularly in *Micrasterias* species, has attracted the attention of cell biologists for many decades. Fascination about the beauty of *Micrasterias* cells can be traced back to the beginning of the 19th century, when the Swedish scientist Agardh found this alga for the first time and described it as "actinomorphous" and "containing a dispersed green powder" (1). Although Agardh classified it into the family Ulvaceae, the first detailed description of the morphology and the taxonomy of the genus *Micrasterias* as a desmid was given in 1848 by John Ralfs (80) in his book *The British Desmidieae*. These early descriptions were followed by the first studies on cell division and observations on cell shape formation by Hauptfleisch (17) and Lütkenmüller (46). The introduction of an appropriate culture medium by Pringsheim (79) and Waris (95) represented the basis for the first experimental studies on morphogenesis, which were carried out essentially by the school of the Finish scientist H. Waris. By focusing on the cytoplasmic processes that lead to the formation of the cell symmetry, Waris (96) postulated the presence of a "cytoplasmic framework" which was thought to be manifested in a system of "cytoplasmic fibrils" arranged as longitudinal axes of the polar and the main lateral lobes. Although this theory has never been verified, the idea of a cytoplasmic determination of the cell shape has remained the subject of various investigations. At the same time as Waris wrote his first articles on morphogenesis in *Micrasterias* cells, Teiling (86) stated in his considerations on the origin of the radiation in desmids that "... The most peripheral layer of the cytoplasm is the form-giving agent ...", an assumption which is close to our present understanding that membrane phenomena are important for cell development.

In subsequent years, vital staining experiments and studies on permeability were used as tools to gain insights into the physiology of a *Micrasterias* cell (7, 20, 26). However, apart from the elegant studies on nuclear control by Waris and Kallio (98), only the introduction of electron-microscopic techniques yielded further information on morphogenesis (see, e.g., reference 10). Kiermayer (29) was the first to investigate different developmental stages of *Micrasterias* cells by using an excellent fixation method and was thus able to show the changes that occur in the cytoplasm during cell development. His conception of *Micrasterias* morphogenesis, based on these early findings as well as on his experiments on turgor-reduced cells, is still valid (27, 29–32). According to Kiermayer, the plasma membrane plays the major role in *Micrasterias* cytomorphogenesis by bearing special "membrane recognition areas" that control fusion of the wall material delivering vesicles.

The aim of the present article is to summarize the various data on cell shape formation that have been collected over the last few years and to combine the single results into a survey of our present knowledge on *Micrasterias* morphogenesis. It will be shown that although many details were elucidated in the past, a wealth of basic questions remain to be solved.

**MORPHOLOGY AND VARIATIONS OF CELL SHAPE**

The *Micrasterias* species that have been used for studies on cell shape formation are the largest and most spectacularly shaped cells within the family Desmidiaceae (Fig. 1a and b). Each cell consists of two semicells with the same size and shape arranged like a mirror image. The two halves of a *Micrasterias* cell are connected by a narrow central constriction, the isthmus. This is also the area where the two cell wall pieces (one being older than the other) overlap. Each fully developed *Micrasterias* semicell has one polar lobe and four main lateral lobes (Fig. 2) that are again indented at their tips. Normal biradiate *Micrasterias* cells are flat and disc-like, with all main lobes developing in one plane. One large flat chloroplast occupies the central area of each semicell and is spread far into the tips of the lobes, its shape thus
FIG. 1. (a) *M. denticulata*. (b) *M. thomasiana*. (c) *M. thomasiana* f. uniradiata. Bar (panels a to c), 50 μm. Panels d to j show the developmental stages of *M. denticulata*. (d) Young bulge stage. (e) Development of the first two indentations (arrows). (f) Development of the second indentations. (g) Five-lobe stage. (h) Five-lobe stage forming the next indentations (arrows). (i) Doubling of the lateral lobes. (j) Formation of further indentations and lobe tips. N, nucleus. Bar (panels d to j), 30 μm.
copying the outer cell shape. At both sides of the chloroplast, large vacuoles fill the body of a nongrowing cell. The nucleus of a Micrasterias cell is large and covers almost the entire isthmus area.

Besides the symmetrical biradial forms, in both cultures and natural habitats, variations of Micrasterias cells that exhibit uni-, tri-, or quadriradial morphology are found (25, 54, 79, 98). These cells are especially interesting for studies on morphogenesis since comparison of their method of cell shape formation with that of the biradial forms can yield valuable information on the basic principles of morphogenesis. The shape of these cell strains seems to be determined by environmental conditions such as composition and concentration of the nutrient solution, which has been demonstrated for one of these shape modifications (13). This latter cell, Micrasterias thomasiana f. uniradiata, has one polar lobe and only two main lateral lobes instead of four in each semicell (Fig. 1c). This uniradiate strain tends to revert to a normal biradial form (76), with the stability of the uniradiate form depending on external factors (13). Several different intermediates between the uniradiate and the biradial forms; with various degrees of differentiation, can be observed (13). Corresponding to the changed morphology, the entire course of cell development as well as single processes such as nuclear migration are also liable to pronounced alterations in these uniradiate cells (54, 55). Since the shape of these morphological variants may change depending on the environmental conditions, they are not regarded as real mutants (13).

**CELL DEVELOPMENT AND ULTRASTRUCTURE**

Mitosis in cultivated Micrasterias cells occurs every 3 or 4 days and is followed by the growth of one half cell, during which the cell pattern has to be reformed.

At an ultrastructural level the onset of mitosis is indicated by the deposition of wall material, which forms a girdle around the isthmus area at early prophase (40). While the nuclear membrane breaks down and mitosis proceeds, a thin septum originating from the cell wall girdle grows inward centripetally like a diaphragm, separating the two half cells by early telophase (Fig. 3). Its growth is mediated by the fusion of "septum vesicles" (11, 32), which are produced by the dictyosomes before and during mitosis and accumulate in the isthmus area. This first step in cell development is one of the most important for morphogenesis. As will be demonstrated below, a prepattern of the later cell shape is already present during septum formation. It may be visualized by means of turgor reduction (28).

Septum formation lasts between 15 and 20 min. Its com-

![Fig. 2. Schematic representation of a growing M. denticulata cell pair. ghc, growing half cell; nghc, nongrowing half cell.](image)

![Fig. 3. Septum formation in M. denticulata. V, vacuole. Bar, 10 μm. Photomicrograph courtesy of Doris Wittmann-Finegger.](image)
pletion is visible in the light microscope by an inhibition of the passage of crystals (BaSO₄ crystals [53]) that were able to move freely within the isthmus before that stage. As soon as the two half cells are separated, primary wall growth starts, with each of the old half cells forming a bulge (Fig. 1d). At the beginning, this bulge grows uniformly in the main level of the cell. After about 75 min, two areas at the cell periphery stop their growth and the first indentations are formed (Fig. 1f to j) until the shape of the growing half cell represents a mirror image of the old cell half. The temporal sequence in which the indentations are formed corresponds to the depth of the indentations in the final cell shape. The sooner an indentation is formed, the deeper it will become. This fact distinguishes some species within the genus Microstera. For example, the difference between M. denticulata (Fig. 1a) and M. thomaisiana (Fig. 1b) lies in the location of the deepest indentations; in M. denticulata the deepest indentations are those between the two lateral lobes, whereas in M. thomaisiana all indentations between the five main lobes are of the same depth. Accordingly, cell development of the two species is also different. In M. denticulata formation of the indentations between the two lateral lobes takes place first, whereas in M. thomaisiana cell shaping starts with a synchronous formation of all four main indentations between the polar lobe and the lateral lobes.

Early studies by Kiermayer (30) showed that at the beginning of cell development the young bulge is essentially filled with vesicles containing either mucilage or primary wall material (see also references 32 and 63). The most prominent structures within a growing Mirostera cell at the stage of primary wall formation are the dictyosomes that are located around the nucleus in the central part of the cell (Fig. 4). Although early studies of chemically fixed cells yielded information about the size and number of cisternae (30, 64), later studies involving high-pressure freeze fixation demonstrated their enormous activity in producing secretory vesicles (Fig. 4) during morphogenesis (61). Dictyosomes in M. denticulata consist of a constant number of 11 cisternae throughout the entire cell cycle, showing a pronounced cis-trans polarity with wide cisternae at the cis side and narrow, heavily stained cisternae at the trans side (Fig. 5). During formation of the protoplasmic primary wall (93), the Golgi stacks produce secretory vesicles that were defined as dark vesicles (DVs) by Kiermayer (30) and contain acid polysaccharides (pectins) (63, 91). Their membranes carry rosettes for microfilament formation (14). DVs seem to go through a developmental process while being forwarded from one Golgi cisterna to the next (61). Their production starts at the fifth or sixth cisterna, whereas the final vesicles that, in size and staining, correspond to those found distributed in the cytoplasm are present only at the trans side of a dictyosome (Fig. 5).

DV s are transported to the plasma membrane by cytoplasmic streaming. Their distribution within the growing half cell appears to be random. Their fusion with the plasma membrane is essential for both expansion of the cell wall and growth of the plasma membrane. However, it has been a point of discussion for many years whether vesicle fusions with the plasma membrane occur over the whole surface of the growing half cell or are limited to the areas of the outgrowing lobes. If the former were true, this would mean that a uniform layer of wall material would first cover the cell surface and that cell ornamentation would be a matter of modulation of this wall material. Outgrowth of the cell lobes could then be explained only by enhanced stretching of the wall in the areas of the lobes as a result of an enhanced plasticity of the wall material. In fact, the primary wall in these areas appears to be thinner than in the regions of the indentations (Fig. 6a).

However, recent results obtained from studies on high-pressure frozen and freeze-substituted Microstera cells make clear that an enhanced deposition of wall material also takes place at the growing tips (61). Figure 6a demonstrates large numbers of secretory vesicles at the tips of the lobes but only a few in the areas around the indentations. Accordingly, the number of vesicle fusions at the growing tips is many times that at the indentations (Fig. 6b and c). These results confirm observations of Lacalli (41), who revealed in autoradiograms that incorporation of [methy]-3H)methionine and [1-14C]glucose into the primary wall is concentrated at the tips of the lobes. The vesicles found to fuse with the plasma membrane at the tips of the lobes belong mainly to the population of DVs. Although they are randomly distributed in the central cytoplasm, they appear to be lined up in the cortical cytoplasm, forming rows oriented perpendicular to the cell surface (Fig. 6b). This may indicate that fusions of DVs occur at presumptive fusion spots in the plasma membrane.

Although DVs preferentially fuse at the growing lobes, another, smaller vesicle population is present, particularly in the areas of the indentations; however, this population is much smaller. It includes vesicles with a diameter of about 70 nm that are found both within the DVs at the growing tips (Fig. 6b) and in the areas of the indentations, where they are frequently seen to be in contact with the plasma membrane (Fig. 6c). Like the DVs, these vesicles appear to be produced by dictyosomes during primary wall formation (61). Their site of formation seems to be the trans-Golgi network, to which they are frequently linked (Fig. 5).

Therefore, there are probably two kinds of vesicles that are building up the primary wall, the DVs and this latter smaller vesicle population that for convenience will be referred to as A vesicles (AVs) below. The studies suggest that DVs are the agents of cell expansion. Where they are incorporated (namely at the growing tips), an extension of the cell wall occurs. The function of the AVs is still unclear, yet several possibilities for their participation in cell shaping may be discussed. One is that since these vesicles are found mainly in the areas of the indentations, they deliver wall material, which, although leading to an increase in the thickness of the primary wall, does not allow a stretching of the wall. This would mean that these vesicles contain wall material that is chemically different from the contents of the DVs. Since AVs have been clearly outlined only recently with the help of cryofixation, no cytochemical analysis has been submitted so far. Another possible function that could be considered for these vesicles is that they represent kinds of "nongrowth markers" maybe containing information that prevent fusions of the DVs in areas where AVs have already fused.

Primary-wall formation in M. denticulata lasts 4 h and 50 min (measured from the completion of septum formation with the help of the cellulase Onozuka test [36]) and is followed by the formation of a rigid cellulose secondary wall. The onset of secondary-wall formation is indicated by the appearance in the cytoplasm of another vesicle type that, like the primary-wall-forming DVs, is also produced by the dictyosomes. These vesicles were first described by Dobberstein and Kiermayer (9) as flat vesicles (FVs). They have a disc-like shape and are characterized by an unusually thick membrane at two sides (Fig. 7a) and sack-like appendages at
FIG. 4. Central area of an *M. denticulata* cell with numerous dictyosomes. The cell has been subjected to high-pressure freeze fixation followed by freeze-substitution (the method is described in reference 61). Ch, chloroplast; LV, large vesicles; M, mitochondrion. Bar, 1 μm.
their edges. The thick membrane domains of the FVs carry globular particles at their inner surface that develop after the vesicles have been pinched off from the trans side of a dictyosome (Fig. 7a to c). FVs fuse with the plasma membrane, with their sack-like appendages becoming attached to the plasma membrane. When an FV opens, the globular particles of its inner membrane surface are incorporated into the plasma membrane. Freeze-etch studies revealed (14, 38) that the globular particles of the FVs correspond to hexagonally arranged rosettes that are present in the plasma membrane during secondary-wall formation (Fig. 7d). Since their distribution is identical to the patterned arrays of the microfibrils in the secondary wall (Fig. 7e), these rosettes are thought to represent enzyme complexes for cellulose formation. According to Giddings et al. (14), each rosette is supposed to produce one 5-nm elementary fibril and one row of rosettes is responsible for the formation of a 35-nm microfibril. Fusion of large numbers of FVs with the plasma membrane seems to account for the typical pattern of crossed microfibril bands, each consisting of about 15 parallel microfibrils, that is characteristic for Micrasterias and other desmids (Fig. 7e) (65-68).

Since a uniform secondary-wall layer copies the shape of the primary wall, secondary-wall formation itself is not regarded as a process of cell modeling. However, during secondary-wall growth, cell wall pores form (Fig. 7e) in a regular distribution over the cell surface (70). Pore formation starts at the beginning of secondary-wall deposition and is mediated by special pore vesicles (PVs) (8) that fuse with the plasma membrane at discrete spots and form plugs within the growing wall. These plugs force the microfibrils to surround them and develop into a differentiated pore apparatus later (70). Cytochemical studies revealed that PVs that still resemble the mucilage-containing large vesicles (LVs) (30) in size and shape contain neutral polysaccharides while the LVs are composed of acid polysaccharides (63). It remains unclear how the hexagonal distribution pattern of the cell wall pores is formed. Some indications for their establishment will be discussed below. As soon as pore formation and secondary-wall growth are completed, slime secretion through the pores starts, initiating the final step of Micrasterias cell development, the shedding of the primary wall (12). It is probably caused by a sudden onset of slime production, which pushes the primary wall away from the cell surface.

The electron-microscopic studies have demonstrated that switching of the dictyosomes to produce different kinds of vesicles during the course of cell growth represents an important structural basis for morphogenesis in Micrasterias cells (Fig. 8). Production of septum vesicles is followed by the formation of primary-wall material containing DVs. Thereafter the dictyosomes give rise to FVs, which deliver enzyme complexes for microfibril formation of the second-
FIG. 6. (a) Lobes of *M. denticulata* at the stage of primary-wall formation, showing vesicle accumulation at the growing tips. Bar, 2 μm. (b) Higher magnification of a growing tip with rows of DVs (arrows) perpendicular to the plasma membrane. Bar, 0.5 μm. (c) Indentation during the stage of primary-wall (PW) formation, with small vesicles (probably AVs) attached to the plasma membrane. Bar, 0.5 μm. Reprinted from reference 61 with permission.
FIG. 7. Panels a to c show FVs with globular particles in the cortical cytoplasm. (a) Lateral section of an FV in *M. denticulata*. Bar, 0.2 μm. (b and c) Tangential sections through FVs of *M. muricata*. SW, secondary wall. Bar, 0.2 μm. Photomicrograph courtesy of Andreas Holzinger. (d) PF face of an *M. denticulata* cell at the stage of secondary wall formation, showing rosettes arranged in a hexagonal pattern. Bar, 0.1 μm. (e) Tangential fracture along the outer surface of the secondary wall of *M. denticulata*, with microfibril bands crossing each other at different angles. P, cell wall pore. Bar, 0.5 μm. (f) Primary wall (PW) and secondary wall (SW) after development in the presence of 3.3 μM amiprophos-methyl (APM). Microfibrils of both cell wall layers are arranged as in untreated cells. Bar, 0.5 μm. Panels d to f reprinted from reference 82 with permission.

ary wall, and to PVs, which form plugs within the growing cell wall. Since all these vesicles are fusing with the plasma membrane, with their membranes becoming incorporated into the plasma membrane, the question of membrane recycling arises. As in other plant cells the amount of membrane being delivered by a huge number of secretory vesicles in a growing *Micrasterias* cell clearly exceeds the requirements for membrane growth. Early studies on chemically fixed cells (30, 74), as well as recent experiments with cryofixation (61), revealed coated (probably clathrin-coated) vesicles and coated pits at the plasma membrane of growing *Micrasterias* cells. In high-pressure frozen and freeze-substituted cells, similar vesicles are also found in connection with the dictyosomes. This suggests that a membrane-recycling process via coated vesicles takes place, perhaps retrieving the membranes directly back to the dictyosomes. However, the number of coated vesicles present in the cortical cytoplasm seems to be too small to account for the entire recycling process in a growing *Micrasterias* cell. Similar considerations have been undertaken by Philips et al. (75) and by Steer (85), who discussed another possible vesicle-free recycling process for higher plant cells.

NUCLEAR MIGRATION

The large nucleus of a nongrowing *Micrasterias* cell is located in the isthmus and is surrounded by numerous dictyosomes and mitochondria. As cell development proceeds, the nucleus migrates into the growing half cell and, at the same time, chloroplast expansion starts (Fig. 1e and f).
The nucleus follows a straight-line path while moving away from the isthmus. After about 1 h, the nucleus stops and returns to its original central position. The removal of the nucleus from the isthmus allows the chloroplast to pass the central narrowing of the cell and to expand into the growing half cell (52). Nuclear and chloroplast migration occur with the participation of cytoskeletal elements.

A microtubule (Mt) center (MC), located behind the nucleus during its migration into the growing half cell and in front of the nucleus during nuclear remigration, represents the initial point for a complex Mt system surrounding the nucleus during its migration (Fig. 9b) (52). The Mt system, which was termed the posttelophase system (PTS) (29, 52), is composed of two components, an Mt cage (Fig. 9a) surrounding the isthmus-facing side of the nucleus and an Mt tail that arises from the MC and is oriented toward the center of the isthmus region. The Mt cage is thought to be functional in holding the nucleus in the proper position during its motion, whereas the Mt tail seems to be involved in orienting the motion. Filamentous structures are visible between the Mts of the PTS. By using microinjection of fluorescence-labeled actin or phalloidin into living *Micrasterias* cells, these filaments were identified as consisting of actin (62). Whereas nuclear migration into the growing half cell seems to be a passive process activated by the turgor pressure and/or the expansion of the chloroplast into the growing semicell, the moving force for nuclear remigration into the isthmus probably arises from Mt-microfilament interaction. A pronounced distortion of the nucleus toward the moving direction indicates that it is pulled backward by a “motor” located in front of it. Particularly, destruction of Mts with Mt inhibitors (see below) results in a deviation of the nucleus from its straight-line path while moving into the growing half cell followed by its dislocation and an inhibition of its remigration toward the isthmus. No further mitosis occurs when the nucleus occupies any other position than the isthmus of the cell (see reference 58 and references therein).

Comparative studies on a uniradiate *Micrasterias* strain that lacks its cell pattern at one side (*M. thomaisana f. uniradiata*) indicated a relationship between the outer cell shape and the course and mechanism of nuclear migration. In this cell the nucleus turns around one side of the isthmus indentation instead of following a straight-line path like in *M. denticulata* when moving into the growing semicell (Fig. 10). Accordingly, the MC in *M. thomaisana f. uniradiata* does not leave the isthmus region during nuclear migration but seems to be connected to the plasma membrane surrounding the isthmus indentation, thus being located asymmetrically within the cell (54). Instead of the Mt tail in a *M. denticulata* cell, a prolonged cortical Mt system (isthmus system of MtS [IS] (29) that is also known from other desmids (see, e.g., reference 56) seems to serve as a guiding element for nuclear motion in *M. thomaisana f. uniradiata*.

Some aspects of nuclear migration, especially of assembly and disassembly of the involved Mt systems, are discussed below.

**EXPERIMENTAL APPROACHES TO *MICRASTERIAS* MORPHOGENESIS**

**Influence of the Nucleus**

There is no doubt that *Micrasterias* morphogenesis is controlled by the nucleus. By eliminating the nucleus with the help of centrifugation (23, 25), local UV irradiation (24, 78, 83), or application of inhibitors of RNA or protein synthesis (15, 72, 87), the transfer of nuclear information for morphogenesis can be interrupted. One of the basic results that we have obtained from these studies is that the basic symmetry of a *Micrasterias* cell is determined before mitosis, which is indicated by the development of a three-lobed semicell even when the nucleus has been removed. This
means that the first morphological features of a growing *Micrasterias* cell, namely three main lobes (one lobe corresponds to the later polar lobe and the two others corresponding to the prospective lateral lobes), form without continuous nuclear control. mRNA and proteins that bear the information for the morphogenetic events are obviously synthesized long before this stage, during the previous interphase. Since no further differentiation of the cell shape occurs without nuclear control (25), the subsequent stages of cell shape formation require additional genetic information.
This is also suggested by experiments with inhibitors of RNA or protein synthesis, which reveal a relationship between cell age at the beginning of the treatment and the cell shape that develops under the influence of the drug. The later protein synthesis is inhibited during cell development, the higher is the degree of differentiation that will be reached. Normal Micrasterias morphogenesis thus requires a continuous nucleus-dependent protein synthesis throughout the entire developmental process (32).

The aberrations that occur when the nuclear influence is eliminated are summarized as anuclear-type development syndrome (36, 83) and are independent of the type of treatment. The most important effects are formation of three to five cylindrical lobes with round tips and without further differentiation, reduction of the polar lobe (see Fig. 16a), an enhanced stretching of the primary wall for up to 24 h that results in a bursting of the cells (51), changes in the ultrastructure of the primary wall (34), an inhibition of both secondary wall formation and production of FVs, and a reduction in the number of dictyosomes (for further details, see reference 36).

Insights into nuclear control of Micrasterias morphogenesis can be obtained not only by decreasing the influence of the nucleus but also by enhancing the nuclear ploidy. Largely because of research done by the group of Waris and Kallio (25, 98), we know about the relationship between an increase of nuclear control, e.g., in binucleate, diploid, or polyploid cells, and the arising cell pattern. Generally, an increased nuclear influence increases the size of the new semicell and leads to a higher degree of differentiation, indicated by an increase in the number of lobes (25). One of the most interesting morphological features, especially in diploid cells, is a doubling of the lobes at their tips. In diploid clone cultures a facies change may take place. This means that the number of main lobes increases, with the additional lobes protruding from the main level of the cell. In this way, triradiate or even quadriradiate cells are formed (for further details, see reference 25). Many questions about how cell shaping occurs in these cells remain to be resolved.

**Visualization of a Prepattern for Morphogenesis by Means of Turgor Reduction**

One of the most significant findings concerning Micrasterias morphogenesis was the visualization of a septum initial pattern by Kiermayer (27, 28), who showed that a prepattern for the later cell shape is already present at this early stage of cell development, prior to any morphogenetic events. With only little technical expense, Kiermayer demonstrated that under turgor reduction, e.g., in a glucose solution, a patterned deposition of wall material occurs. It is characterized by one central and two lateral minimum zones (see below), corresponding to the lobe indentations that will form during the later developmental sequence (Fig. 11a). Thus a template for morphogenesis present at the early stage of septum growth already contains the information for the basic symmetry (a three-lobed structure) of a Micrasterias cell. Kiermayer (31, 32) assumed that the septum initial pattern is determined by the plasma membrane, which probably bears membrane recognition sites (receptors) for the membranes of the secretory vesicles. During turgor reduction, these vesicles fuse with the plasma membrane at local areas without being properly incorporated into the cell wall. This leads to characteristic patterned accumulations of wall material. The way in which the pattern of membrane recognition sites comes into the plasma membrane during septum growth is still the subject of speculation. Since we know that fusion of septum vesicles accounts for both septum wall formation and extension of the surrounding plasma membrane, these vesicles would be candidates for supplying the requisite information, perhaps in the form of macromolecular proteins or ion channels (see below). However, the question arises of how these vesicles are guided to their...
proper position at the growing septum such that their membranes can establish a pattern. One possibility that could be considered is that different kinds of septum vesicles (perhaps with different membrane compositions) are produced in a temporal sequence by the dictyosomes. Since septum formation starts at the margin of the isthmus and proceeds toward the center, this would mean that a sequence of fusions of different vesicle populations would cause the formation of a simple pattern. Another possibility would be to postulate a kind of self-assembly process as the basic mechanism for template formation. However, *Micrasterias* morphogenesis seems to be too complicated to be explained by such a basic molecular mechanism. Lacalli (42) suggests that the shape of the isthmus acts in part as a template for *Micrasterias* morphogenesis, possibly by determining its radial symmetry. However, he does not explain how the basic symmetry that, in his opinion, is manifested in the shape of the isthmus is realized during morphogenesis of a growing cell.

Mts can be eliminated from being involved in the guidance of septum vesicles, since the cortical Mt system (IS) breaks down before septum formation starts. Microfilaments are probably important for transporting septum vesicles to their prospective fusion sites. Their disruption by cytochalasin B results in an inhibition of septum formation (43). However, it remains to be investigated to what extent actin filaments participate in pattern formation (see below).

Wall material accumulations produced by turgor reduction can also be used to visualize the growth areas of a *Micrasterias* cell in the successive stages of patterned primary-wall formation. Experiments show that each developmental stage is characterized by zones that are able to accumulate great amounts of wall material (maximum zones), whereas in other areas (minimum zones) little or no wall material accumulates (Fig. 11b). Careful studies by Kiernayer (27) show that the minimum zones represent the prospective lobe indentations whereas the maximum zones correspond to the prospective lobes (i.e., the actual growth areas) (Fig. 12). It is interesting that the pattern visualized by means of turgor reduction represents a prepattern for a morphological event that takes place later in cell development. As with septum formation (see above), a minimum zone for a discrete indentation can be visualized some time before the indentation starts to be formed. This again supports the assumption expressed by Kiernayer (31, 32) that the plasma membrane contains a kind of template for *Micrasterias* morphogenesis.

**Distribution and Function of Membrane-Associated Calcium and Ionic Currents during Morphogenesis**

Ca$^{2+}$ ions are known to regulate morphogenetic events by controlling localized exocytosis in various tip-growing plant cells (see, e.g., references 18 and 84). In *Micrasterias* cells the distribution pattern of membrane-associated Ca$^{2+}$ visualized by application of chlortetracycline (49, 50) changes continuously during cell development. According to the cell pattern that becomes more and more differentiated during cell growth, the calcium accumulation zones, which are limited to one or three areas at the beginning of cell development split several times (Fig. 13a to c). The spatial distribution of membrane-associated Ca$^{2+}$ corresponds to the pattern of the actual growth areas in each developmental stage.

Comparative studies with *M. thomasianna f. uniradiata* (see above) clearly revealed a correlation between calcium accumulation and pattern formation (55). Whereas the side of the cell that carries the typical cell pattern also exhibits fluorescent areas after chlortetracycline treatment, no Ca$^{2+}$ signal is found on the other (nonornamented) side (Fig. 13d). This means that outgrowths of the lobes of a *Micrasterias* cell occur only at areas characterized by an accumulation of membrane-associated calcium. These results are supported by recent studies in which one- and two-dimensional vibrating probes were used to measure transcellular ionic currents in growing *Micrasterias* cells (88–90). Inward ionic currents that “may be carried at least in part by Ca$^{2+}$, Cl$^-$, H$^+$ and K$^+$ ions” (89) enter at the local areas of cell expansion and exit at the areas of the cell indentations and at the surface of the non-growing semicell (Fig. 14). Outward currents are also detected at the nonpatterned side of *M. thomasianna f. uniradiata* (Fig. 15). Treatment with Ca$^{2+}$ channel blockers such as gadolinium results in a decrease of the measured currents (89). Accordingly, elimination studies with different salt solutions (47, 97), as well as experiments with ethylene glycol-bis[β-aminoethyl ether]-N,N,N',N'-tetraacetic acid (EGTA) and LaCl$_3$ (45), show that Ca$^{2+}$ is required for normal completion of morphogenesis at least in trace amounts. In the presence of the ionophore A23187 and CaCl$_2$ in the nutrient solution, cell growth is inhibited. Although no direct influence on morphogenesis can be observed in these cells, the accumulation pattern of wall material obtained by turgor reduction seems to be changed (47). It appears to be more uniform than in cells grown without the ionophore. However, since the pattern is not completely extinguished by the ionophore, the studies do not show clearly whether the ionophore treatment equilibrates a Ca$^{2+}$ gradient within the cell (81), leading to an uniform Ca$^{2+}$ influx over the growing half cell. This, however, might be expected to result in an entirely uniform unpatterned deposition of wall material. The relatively thin wall layer acquired by cells exposed to the ionophore and the less-pronounced wall material accumulations could also be due to an inhibition in the production of secretory vesicles and do not necessarily reflect a specific effect of the ionophore on pattern formation. This is also suggested by the observation that secondary-wall formation does not take place during treatment with the ionophore (47). Since it is known that ionophores may
disorganize dictyosome structure and activity in various plant cells (see, e.g., reference 69), the lack of a secondary wall in ionophore A23187-treated Micrasterias cells could be explained by an inhibition of FV production. McNally and Swift (48) report a dispersion of DVs as a consequence of ionophore A23187 treatment. However, there are no data on the structure and activity of the dictyosomes and on the number of vesicles produced during drug treatment.

Nevertheless, there is no doubt that calcium plays a major role in Micrasterias morphogenesis. As stated in various review articles (16, 19, 84), the action of calcium in cell growth may be diverse. According to Jaffe (21, 22), an accumulation of calcium channels in the actual or prospective growth areas generates an electric field by increasing the Ca$^{2+}$ influx. The resulting calcium concentration gradient is supposed to lead to a localized deposition of wall material by attracting cell wall material containing vesicles. By using these assumptions for Micrasterias cells, this means that the number of calcium channels at the areas of the outgrowing lobes is larger than in between. This is in good agreement with the finding that inward ionic currents are detectable only in the areas of cell expansion (89; see above). Application of external electric fields obviously destroys the endogenous electric field and cause severe aberrations of the cell shape (6). The density of membrane particles, determined in freeze-fracture studies to be significantly higher at the growing tips than in other regions of a Micrasterias cell (5), is changed under the influence of an applied electric field. The calcium channels that are postulated to concentrate at the growing tips may be delivered by the membranes of primary wall material containing DVs that preferentially fuse in these areas (see above), thus further amplifying local wall material secretion, as has been suggested for other cells (3, 16). It is still unclear whether calcium currents regulate morphogenesis or whether their restriction to local areas is a consequence of morphogenetic events that occurred earlier in cell development. To determine this, calcium currents would have to be measured before and during the very first step of cell development, septum growth.

The considerations discussed above can also be used to address pore formation in Micrasterias cells. Local accumulation of calcium, limited to small spots that are distributed in a particular pattern over the inner cell surface, has been demonstrated to precede pore formation (Fig. 13d) (50). These localized areas of membrane-associated calcium seem to determine the distribution of cell wall pores by controlling the fusion of pore vesicles. The calcium distribution pattern at the beginning of secondary-wall formation is completely different from that characteristic of the final step of primary-wall formation (35; see above). Therefore, a sudden change in the calcium distribution seems to occur at the moment when primary-wall formation stops and deposition of the secondary wall starts. The studies of pore formation show that the function of calcium in cell development is not necessarily limited to mediating processes that are connected to cell expansion but seem to lie primarily in controlling fusions between vesicles and the plasma membrane.

Calcium may also effect a cortical actin network that may function in localized vesicle fusion. Since cytochalasin B causes cell shape malformations when applied during the early developmental stages and prevents further cell growth, an involvement of actin filaments in morphogenesis seems to be likely (44, 73, 87). This is also suggested by cytochemical and electron-microscopic studies exhibiting actin cables in chemically fixed developmental stages and nongrowing Micrasterias cells (74, 92; see below).

FIG. 13. Distribution of membrane-associated calcium visualized with the help of chlortetracycline fluorescence. (a to c) Developmental sequence of M. denticulata with splitting of the fluorescent areas corresponding to the cell pattern. (d) M. denticulata at the stage of pore formation. Fluorescent dots corresponding to the distribution pattern of the cell wall pores are visible (arrows). (e) Young developmental stage of M. thomassana f. uniradiata, with fluorescent areas only at the side which develops a normal cell pattern. (f) Developmental stage of M. denticulata grown at 35°C. Fluorescence is visible only between the outgrowing lobes. Reprinted from references 49 (a to c), 50 (d), 55 (e), and 57 (f) with permission.
Shape Aberrations after Physical Treatment

Interesting results on morphogenesis were obtained in studies in which a laser microbeam or UV light was used to irradiate certain areas of the cell periphery of growing Micrasterias cells (41, 71, 83). Nishimura and Ueda (71), who were able to localize the microbeam to special areas of the plasma membrane, showed that irradiation of points at a growing tip causes an inhibition of growth in the relevant area and leads to marked morphological changes. In contrast, after irradiation at the region of an indentation, cell development occurs quite normally. This again supports the assumption (32) that particular specialized areas of the plasma membrane control Micrasterias morphogenesis (see above). The size of these areas has been demonstrated to measure about 4 to 5 μm in the later stages of cell development, when the main lobes have already formed (41). In earlier stages the plasma membrane areas that are important for morphogenesis cover no less than 10 μm in diameter. A further indication of the mechanism of morphogenesis arises from the fact that irradiation of the growing cell wall (without irradiating the adjacent cytoplasm) also causes shape aberrations. Therefore, special properties of the primary wall, possibly enzyme activities that increase the plasticity of the cell wall, are required for normal cell shaping, in addition to the events at the plasma membrane (see above).

Centrifugation experiments during primary-wall growth in a Micrasterias cell reveal a dislocation of secretory vesicles, leading to shape malformation. Whereas DVs are removed from the cortical cytoplasm at the centripetal side of the cell, a layer of LVs prevents their fusion with the plasma membrane at the centrifugal side (33). Both of these events lead to a local inhibition of cell growth. Since the centrifugal force counteracts the turgor pressure, accumulations of cell wall material similar to those found after turgor reduction appear in the areas of inhibited growth. It remains to be investigated to what extent the forces that become effective during centrifugation act on the cytoskeleton system (e.g., on actin microfilaments) that may hold the secretory vesicles in their position, respectively cause their motion.

Long-term centrifugation during the entire growth process results in the formation of small, undifferentiated semicells. These cells maintain their ability to divide and produce small
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**MICRASTERIAS** cells that regain their normal shape during further cell divisions (33).

Cell shape formation in **MICRASTERIAS** cells is also influenced by the temperature. Although nongrowing cells exhibit a considerable tolerance to temperature changes (4), cells at the stage of primary wall formation respond to small temperature changes with dramatic shape aberrations (57). Generally, lowering the growth temperature leads to a retardation of growth and to a simplified cell pattern. When the temperature is increased from 20 to 34 to 36°C, the situation is more complicated. It may likewise lead to a simplification of cell ornamentation (Fig. 16b), as well as to the formation of semicells with an increased number of main lobes protruding from the normal level of the cell and covering a relatively large undifferentiated cell body (Fig. 16d). In most cases the cell symmetry of these aberrant cells is lost (Fig. 16c). The malformations of the cell shape induced by high-temperature treatment are combined with a shift in the distribution of membrane-associated calcium (57). In contrast to cells grown at normal temperature (20°C [see above]) Ca²⁺ accumulation areas are localized between the outgrowing lobes in these cells (Fig. 13f). Accordingly, growth of the lobes seems to be a matter of stretching of the cell wall rather than of additional deposition of wall material. This becomes obvious at an ultrastructural level, at which the cell wall in the areas of the lobes appears to be extremely thin.

It is known that temperature changes influence membrane properties. Since the elevated temperatures used to produce malformations in **MICRASTERIAS** cells are too low to affect protein structure (see, e.g., reference 2), a change in the lipid content or the lipid protein interaction in the plasma membrane may account for the change in the cell pattern. Additionally, high temperature increases the amount and length of endoplasmic reticulum cisterna and influences protein synthesis by leading to the appearance of heat shock granule aggregations in the cytoplasm of growing **MICRASTERIAS** cells (for details and references, see reference 57).

### Role of Microtubules and Microfilaments in Cell Development

Early studies by Kiermayer (29) revealed four Mt systems in a growing **MICRASTERIAS** cell. Clusters of ring-shaped Mts (IS) are present in the cortical cytoplasm of the isthmus. The IS is extended in both half cells in interphase, with the number of Mts decreasing with the distance from the isthmus. The IS breaks down during mitosis and is reestablished in the nongrowing semicell after mitosis, whereas in the growing half cell only a few Mts are found in the isthmus area (52). As soon as cell shape formation is completed and the nucleus has reoccupied its position in the isthmus, the IS regains its interphase form in both half cells. The function of the IS is not yet clear. On the one hand, it is thought to be involved in the determination of the plane of cell division, thus operating like a preprophase band in higher-plant cells (30). On the other hand, it may also function in anchoring the nucleus in its central position (52) and participating in chloroplast separation (60).

The most prominent Mt system in a growing **MICRASTERIAS** cell is the PTS around the moving nucleus, which is described above (see the section on nuclear migration, above [Fig. 9a and b]). It arises from an Mt center that, as in other desmids (see, e.g., reference 77), probably originates from the former spindle pole. It is present only during nuclear migration and seems to be responsible for the orientation of nuclear motion and for providing the motive force, especially for nuclear remigration back toward the isthmus. This becomes obvious from studies with Mt inhibitors, which demonstrate that destruction of the Mts leads to complete inhibition of the backward movement of the nucleus (for a summary, see reference 58). The nucleus stays at any position in the cell periphery, and the cell dies after some time. Recovery experiments revealed that destruction of the Mts is reversible (52). Only a few hours after the anti-Mt drug has been removed, the PTS is reestablished (Fig. 9c). Its ability to move the nucleus back to the isthmus seems to depend on the orientation of the Mts. If the Mt tail of the PTS (see above) is oriented toward the center of the isthmus, a slow nuclear remigration takes place. This occurred in about one-third of the cells investigated (52). In the remaining cells the PTS exhibited other orientations and the nucleus was pulled to one of the indentations that lie next to it. When it contacted this indentation, the PTS disintegrated and an abnormal ring-shaped IS was established around the indentation (52). The studies suggest that the nucleus itself or discrete areas of its membrane initiate Mt reassembly. Mts are found only around indentations in the presence of the nucleus and never in untreated cells in which the nucleus is located in the cell center. As a result of the recovery experiments and of studies of untreated cells, it is obvious...
that the PTS and the IS may be converted into each other. Thus, the pool material for both Mt systems seems to be the same.

Dislocation of the nucleus induced by Mt inhibitors is easily to observe in *Micrasterias* cells even at low light-microscopic magnification. Since the Mt system involved in nuclear migration is extremely sensitive to Mt-disrupting agents, even at low concentrations, this advantage has been used to establish *Micrasterias* cells as test objects for identification of anti-Mt agents (59).

Besides the PTS and the IS, two more Mt systems are detectable in a growing *Micrasterias* cell (29). They include randomly oriented Mt s in the central cytoplasm of the growing semicell and in the cortical cytoplasm of both the growing and the nongrowing semicell (29, 74). The first are thought to be involved in chloroplast migration and spreading, but the second group was originally suspected to function in morphogenesis or microfibril deposition of the cell wall (29). However, various investigations have shown that cell shape formation occurs normally even when the Mt s are destroyed (for a summary, see reference 32). Moreover, a recent freeze-etch study has shown that the patterned distribution of the microfibrils in the secondary wall (39) also occurs without participation of Mt s (Fig. 7f) (82). The latter study suggests that the function of the cortical Mt s may be simply to exert a stabilizing effect.

The role of actin microfilaments in *Micrasterias* cells, although probably much more important for morphogenesis than that of the Mt s, is far from being understood. Although electron-microscopic studies with conventional chemical fixation only occasionally revealed filamentous structures between the Mt s surrounding the nucleus, cortical filaments have been depicted after cryosubstitution following plunge-freezing (74) and with the help of fluorescence-labeled phalloidin applied to chemically fixed cells (92). Only recently has evidence for cortical actin in living and growing *M. denticulata* cells been obtained (62). Microinjection experiments with fluorescence-coupled phalloidin in cells at different developmental stages resulted in the appearance of a dense network of actin filaments covering the surface of both the growing and nongrowing semicell.

Since cytochalasin B inhibits cytoplasmic streaming and has cell growth as a consequence when used in high concentrations and leads to severe malformations of the cell shape when applied in low concentrations (44), an involvement of actin in *Micrasterias* morphogenesis is obvious. However, as in other organisms, the question of which processes are the targets for actin filament action still remains. Some authors have postulated that at least the transport of the wall-forming vesicles from the dictyosomes to the plasma membrane is an actin-mediated motion (92). This implies that inhibition of actin microfilament function as a consequence of cytochalasin B treatment prevents further wall secretion and leads to inhibition of cell expansion. However, the occurrence of cell deformations as reported by Noguchi and Ueda (73) and Lehtonen (44) cannot be explained only by absent or reduced cytoplasmic streaming but suggests another more specific function for actin in morphogenesis. This could be, for example, the guidance of vesicle fusions at discrete areas of the plasma membrane. High-pressure freeze fixation studies have revealed that the secretory vesicles in *M. denticulata* line up in front of their prospective fusion spots, forming rows of three or four vesicles perpendicular to the cell surface (Fig. 6b). Actin filaments may be involved in this patterned arrangement and may also be responsible for the distribution of the fusion sites which determines morphogenesis. The cortical location of the actin network visualized after microinjection of phalloidin into growing *Micrasterias* cells suggests a possible interaction with the plasma membrane.

**BASIC QUESTIONS ABOUT MICRASTERIAS MORPHOGENESIS**

There is no doubt that the shape of a *Micrasterias* cell is determined by genetic information. The nucleus controls the basic symmetry of the cell before mitosis, and its molecular products are required for the entire developmental process. However, genetic information can only provide a plan for morphogenesis, whereas the morphogenetic events are expressed on a cytoplasmic level. Therefore, we must focus on changes in the cytoplasm and its membranes that occur during cell development to obtain a better understanding of *Micrasterias* morphogenesis.

*Micrasterias* morphogenesis may be essentially reduced to two problems that seem to be the key events. These are the formation of the septum, during which the first prepattern of the latter cell shape is formed, and the splitting of the lobes, which occurs repeatedly during the course of morphogenesis. Unfortunately, these two processes are the subjects of many unsolved questions and are far from being understood.

In regard to septum formation, the following questions arise. How does the prepattern for morphogenesis get into the septum membrane? What guides the septum vesicles to their proper position? How can a pattern (which is invisible under normal conditions) arise within a few minutes by fusion of a special vesicle type? As in other developmental processes, the two possible candidates that may be considered to control septum formation are ionic currents (e.g., inward ionic currents carried by calcium in a distribution that corresponds to the septum initial pattern) and actin microfilaments.

An additional candidate, i.e., that different populations of septum vesicles are produced in a temporal sequence by the dictyosomes and are incorporated one after the other to produce a simple pattern, is possible, but it would only displace the problem to an earlier stage of development.

With our present stage of knowledge, it is impossible to make any decision about which of the addressed methods is more likely, and it cannot even be excluded that there are other, unknown, factors controlling septum formation.

The first splitting process in a growing *Micrasterias* cell occurs in a way that reflects the septum initial pattern (27) and leads to a three-lobed developing semicell. Formation of the two first indentations and also of the following ones takes place by a simultaneous sudden halt of cell growth at particular areas of the cell edge. Again, this event is completely obscure, and many questions remain to be answered. For example, it is not known what determines the accurate location of these indentations and how this local growth cessation is mediated. The only answer that can be given at present is that the location of the first indentations seems to be predetermined at the septum membrane and can be visualized as minimum zones by means of turgor reduction (see above). When the septum bulges and becomes the primary wall, these minimum zones become localized at the edge of the growing bulb at a defined distance from one another. What happens at the minimum zones during formation of the indentations? Electron microscopy of cryofixed cells revealed the presence of a particular vesicle type (AVs) in the areas of the indentations, while the vesicles known to deliver wall material (DV s) are accumulated in the growing
tips. Assuming that the plasma membrane controls the vesicle fusion processes (which seems to be likely from various experiments [32, 37]) this would mean that the areas of the prospective indentations suddenly lose their activity to attract DVs and favor fusions of the AVs instead. This again could result in changed wall properties at the indentations. It is interesting that once growth has stopped at one point during Micrasterias morphogenesis, no subsequent extension of the cell wall occurs in this area, although the wall increases in thickness. However, as long as we do not have detailed data on the chemical composition of the contents of DVs and AVs, these assumptions remain rather speculative. They also do not explain what causes the proposed change at the minimum zones of the plasma membrane or how the spacing between the further indentations is determined.

One interesting model for explaining the successive branching events that occur during Micrasterias morphogenesis has been suggested by Lacalli and Harrison (43). It is based on a linear functional relationship between temporal and spatial scales during the cell-shaping process and suggests a reaction-diffusion mechanism for control of cytomorphogenesis. Although this model might lead to a better understanding of cell modeling, it does not give much insight into the cytoplasmic events that are responsible for pattern formation.

When regarding the formation of indentations in Micrasterias cells, one should keep in mind that cessation of growth is only one “strategy” to form clefts in desmids. Url and Kiermayer (94) showed that in Euastrum cells, which are closely related to Micrasterias cells, formation of indentations and depressions of the cell surface is caused by enhanced primary-wall growth. The primary wall forms deep conical protuberances by the turgor pressure, and these conical protuberances are surrounded by a secondary wall later in cell development. When the primary wall is shed, the cones are pushed off as well and the clefts become visible. It is more obvious in Euastrum cells than in Micrasterias cells that the wall material building up the cones for the indentations is different from that undergoing extension growth (94).

Since development in Micrasterias species and other desmids is an ideal process for studying morphogenesis in a plant cell and since various data have already been collected over the last few years, efforts should be made to obtain further insights into the two basic processes that underlie cell shape formation. Septum growth and lobe splitting should be influenced more precisely by using ionophores, ion channel blockers, calcium buffer injections, and agents that affect the actin cytoskeleton. Moreover, comparison between the course of cell development in closely related cells that exhibit different cell patterns seems to be appropriate to elucidate morphogenesis.

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