Introduction

The general intent of this collection of chapters is to stimulate the investigation of algal biology and to recommend them as uniquely suitable experimental systems. It is hoped that the volume will aid students of phycology in applying new techniques and will entice experimentalists to explore algal material. It is an introduction to systems and methods from which the investigator can begin.

Chapters are grouped into two general categories. One deals with algae as developmental organisms. The second primarily covers microscopic tools for the study of living cells and the preparation and staining of fixed cells. Generally, the chapters reflect the present state of the art and thus are of necessity variable in content and format. Some will be adaptable for classroom exercises, whereas others require considerable sophistication in application. As introductory chapters they are not intended to be comprehensive. References to more advanced methods are given in most chapters, and a few additional references are included below.

In the chapters, a single species, or a group of species, are used as examples to which the methods have been successfully applied. The species, when appropriate, are identified with a source number of a culture collection listed in the Culture Collections appendix.

Sources of materials and equipment included in the chapters and appendix are for reference only and should not be construed as endorsements. Lists of suppliers are published annually in the United States in Science (American Association for the Advancement of Science, 1515 Massachusetts Ave., N.W., Washington, D.C. 20005); in Canada in Research and Development (MacLean Hunter, 418 University Ave., Toronto 101, Ontario) and Laboratory Products News (Southam Business Publications, Ltd., 1450 Don Mills Rd., Don Mills, Ontario).

General references

Introduction

Section I

Experimental algal systems and techniques
1: Control of morphogenesis in Micrasterias

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I. Objective

*Microstriae*, a unicellular green alga, is an ideal choice for experiments in cell biology and development because of its relatively large size, distinctive shape, and the ease with which it can be cultured and experimentally manipulated. Apart from the opportunity it offers to study cytomorphogenesis, the differentiating cell may be used as a test object for detailed examination of such organelle systems as the Golgi complex and the diverse microtubule systems and their relation to cell function and development. Physiological and ultrastructural studies of the different stages in cell development (nuclear division, septum formation, cell wall growth, etc.) are possible because these developmental events occur consecutively in the cell cycle.

II. Test organism

A. Cytomorphology

The genus *Microstriae* is classified in the family of Desmidiaceae. In the system of Frisch (1961), Desmidiaceae are considered a suborder—Desmidiodae (placoderm Desmids)—of the Conjugales. Our test object, *Microstriae denticulata* Bréb, a medium-sized species, will typically be 180–300 µm long, 165–300 µm wide, and 55–62 µm deep (Krieger 1937). The cell is organized in two semicells with a connecting region known as the isthmus. Both semicells are divided into one polar lobe and several lateral lobes. The nucleus, having a diameter of ca. 30 µm, is located in the isthmus.

B. Cytodifferentiation

1. Nuclear division. Algae ready for cell division are easily detected by their unusually dark, green chloroplast that tends to retract from the isthmus region, their expanded nucleus, and the changed appearance of the nucleoli. The changes seen in the nucleus upon division are described in detail by Waris (1950).

2. Septum growth. The septum grows centripetally so as to divide the two semicells. Cessation of free movement of crystals between the
1. Morphogenesis in Micrasterias

Fig. 1–1. Developmental sequence of the growing semicell of *Micrasterias denticulata*, time interval between pictures is 15 min (20° ± 1°C) made in a flow-through chamber (1 drop/min of medium) (Kiernayer 1964).

Semicells indicate when the septum closes. During septum formation, a nucleus re-forms in both semicells, and a slight extension of the two semicells occurs in the isthmus region.

3. Cytomorphogenesis. Two small hemispherical bulges begin to form after the separation of the semicells by the septum (Fig. 1–1). The bulges are surrounded by a rather fine primary wall. It is in this region that further growth and differentiation of the new semicells is
observed. As illustrated in Fig. 1–1, the hemispherical bulges pucker in the next stage of growth (three-lobed stage) into three lobes. The substructuring to a greater degree of lobing is illustrated in Fig. 1–1 A–P. After the primary wall is completed, a stronger and more rigid secondary wall forms. Swelling and subsequent shedding of the primary wall then occurs. The secondary wall, when formed, contains the pores and pore apparatus. The complete developmental process has been documented cinematographically (Kiermayer 1966).

During the development of hemispherical bulges, vigorous cytoplasmic streaming can be observed. Initially, the developing bulges contain only protoplasm. The nucleus does not enter until the three-lobed stage. At that time, the nucleus and an aggregation of dictyosomes surrounding it are especially conspicuous.

III. Culture methods

A. Growth conditions

1. Growth of natural isolates. The algae are taken to the laboratory from their natural habitat (peat bog). Samples may be kept in peat-bog water in unplugged flasks for several months in a relatively cool place (16–20°C). This can be in a window or on a cool sink, but not in direct sunlight. To obtain semisterile cultures of *Microasterias*, a washing process is carried out under aseptic conditions. With the aid of a stereo-microscope (×18 magnification) *Microasterias* cells are transferred into small petri dishes (6 cm diameter) containing 10 ml of medium. After six such transfers, the cells are inoculated into Erlenmeyer flasks with sterile micropipettes (see below).

Cultures of several species of *Microasterias* are available from the Culture Collection of Algae, University of Texas (UTEX).

2. Defined growth medium. The growth medium employed is essentially the one described by Waris (1950): 0.10 g KNO₃, 0.02 g (NH₄)₂HPO₄, 0.02 g MgSO₄·7H₂O, 0.05 g CaSO₄, and 0.001 g FeSO₄ dissolved in 1000 ml doubly distilled water. Instead of FeSO₄, 5 ml of an iron stock complex may be added to the medium. It is prepared as follows: 0.4 g FeSO₄·7H₂O plus 0.53 g Titriplex III (Merck) is dissolved in 160 ml of doubly distilled water, brought to a boil, and made up with water to 200 ml. The pH of the medium is 5.3.

Flasks (100 ml) are filled with 30 ml of medium, plugged with cotton, wrapped in foil, and steam-sterilized at 100°C for 1 h. After cooling, each flask is inoculated with 10–20 cells (bacteria are not fully excluded) and placed in a constant-temperature (20°C ± 1°C) mirror incubator. The incubator is illuminated at 1,000 lux (95 ft·c) with 3 neon lamps (Osram-L, 40 W) which are vertically mounted in the
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middle. The flasks are placed at a distance of 25 cm from the lamps. Cultures should be transferred every 4–5 weeks, because cell defects increase and cell division rates decrease with the age of the cultures.

3. Light regime. The light-and-dark regime, which is controlled by an automatic clock, is adjusted so that formation of the new semicells occurs between 8 and 10 a.m. The light is on from 12 p.m. to 2 a.m. and off from 2 a.m. to 12 p.m. (Kiernayer 1964).

B. Induction of cell division

The time of the day for the onset of mitosis can be controlled by regulation of the light regime (see above). The normal daily rate of division is only 3% in 3- to 6-week-old cultures. This can be increased to ca. 8% by the light-shock method (Kiernayer 1970a) as follows: The day before use, 3- to 4-week-old Microasterias cultures are shaken and transferred to petri dishes. These petri dishes are then exposed to continuous illumination of 1100–1400 lux (100–130 ft·c). Under a cyclic light regime, cell division occurs between 8 and 10 a.m. in the dark. Under continuous illumination, cell division also occurs between 8 and 10 a.m.

IV. Experimental procedures

The developmental stages, as identified in Fig. 1–1, can be obtained from stock cultures. Starting with septum formation, the time course of development can be predicted as outlined in Table 1–1. Once initiated the developmental stages are completed either in continuous light or in variable light–dark cycles, provided the temperature is maintained at about 20°C.

A. Microscopic observation of developmental stages

1. Cells from a stock culture emptied into a petri dish (6-cm diameter) are selected under a stereomicroscope.

2. With a micropipette 3–4 cells are then placed in a drop of peat-bog detritus (pH 6–7) on a microscope slide (previously washed in a chromate–sulfuric acid solution and thoroughly rinsed). The detritus is required for continued growth and development under a sealed coverslip (Kiernayer 1964, 1966).

3. A clean coverslip is carefully placed over the preparation, which is then sealed with petroleum jelly. Care should be taken to have a thin detritus layer.

4. Observation can be made under low light, sufficient for observation. The temperature around the specimens should not exceed 22°C.

5. Long-term observations can be made with such a setup. It is suit-
### Experimental algal systems and techniques

#### Table 1-1. Time course of developmental processes in *Micrasterias denticulata* *Breb* cells, starting with septum formation

<table>
<thead>
<tr>
<th>Hours</th>
<th>Process Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Septum formation</td>
</tr>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Primary wall formation (cytomorphogenesis)</td>
</tr>
<tr>
<td></td>
<td>Nuclear migration</td>
</tr>
<tr>
<td>3</td>
<td>Chloroplast migration</td>
</tr>
<tr>
<td>4</td>
<td>Beginning of pore formation</td>
</tr>
<tr>
<td>5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Secondary wall formation</td>
</tr>
<tr>
<td>9</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Formation and differentiation of pores to complex pore</td>
</tr>
<tr>
<td></td>
<td>apparatus</td>
</tr>
<tr>
<td>14</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Shedding of the primary wall</td>
</tr>
<tr>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

Able for time-lapse photography as in Fig. 1–1 where each frame represents a 15-min interval.

#### B. Hanging-drop method

With the hanging-drop method, the selection, microscopic observation, and micrography of developing cells can be carried out as above except that the medium with the cells is placed on a coverslip and sealed with petroleum jelly to a depression slide. This setup can be used for special applications, such as testing the effect of inhibitors and narcotic agents that are soluble in water. The modification is as follows:

1. Cells are transferred to the surface of a clean coverslip somewhat larger than the well of the depression slide.
2. The adhering medium is absorbed with a strip of filter paper placed as far away from the cells as possible.
1. Morphogenesis in Micrasterias

Table 1–2. Developmental studies in Micrasterias

<table>
<thead>
<tr>
<th>Cell structure or developmental event</th>
<th>Investigation on</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear division</td>
<td>Chromosomes, microtubules</td>
<td>Waris (1950), Ueda (1972)</td>
</tr>
<tr>
<td>Primary wall</td>
<td>Growth; influence of turgor-pressure; “D-vesicles”</td>
<td>Kiernayer (1964, 1970a), Lacalli (1975)</td>
</tr>
<tr>
<td>Cytomorphogenesis</td>
<td>Formative events; effects of the nucleus and RNA on cell form</td>
<td>Waris (1950, 1951), Kallio (1951), Waris and Kallio (1964), Kiernayer (1964, 1966), Selman (1966)</td>
</tr>
<tr>
<td>Postmitotic nuclear migration</td>
<td>Microtubular systems</td>
<td>Kiernayer (1968, 1972)</td>
</tr>
<tr>
<td>Secondary wall</td>
<td>Formation of microfibrils; formation and incorporation of “F-vesicles”</td>
<td>Mix (1966), Dobberstein (1973), Kiernayer and Dobberstein (1973)</td>
</tr>
<tr>
<td>Slime secretion</td>
<td>Production and incorporation of “L-vesicles”</td>
<td>Kiernayer (1970a)</td>
</tr>
<tr>
<td>Golgi system</td>
<td>Structure of dictyosomes; vesicle production; dictyokinetics; ultra-cytchemistry</td>
<td>Drawert and Mix (1961), (1963), Kiernayer (1970a,b, 1977), Kiernayer and Dobberstein (1973), Menge (1976), Ueda and Noguchi (1976), Menge and Kiernayer (1977)</td>
</tr>
</tbody>
</table>

3. The cells are quickly rinsed with a drop of the test solution and are again quickly covered with a drop of the same solution.

4. The time, starting with the first application, is recorded with a stopwatch.

5. The coverslip is carefully turned over, placed on the depression slide, and sealed with petroleum jelly. Observations can be made over a period of hours or days.

Step 3 requires modification if a volatile solution is to be tested. The cells are suspended in a drop of the volatile solution, and the well of the depression slide is filled with the volatile test solution to within 2 mm of the upper surface. Coalescence with the drop should be