HANDBOOK OF PHYCOLOGICAL METHODS

DEVELOPMENTAL AND CYTOLOGICAL METHODS

EDITED BY
ELISABETH GANTT

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Editor's preface

This handbook on *Developmental and Cytological Methods* is the third of a series sponsored by the Phycological Society of America. It follows the original intention of the first P.S.A. editorial committee constituted in 1967. The first volume, edited by Janet R. Stein, set the standards and general format for the subsequent volumes. It is hoped that this volume has not deviated too far from the high standard set by Professor Stein. Her advice and guidance in the preparation of this volume are greatly valued and appreciated. This volume, along with the first by Stein and the second by Hellebust and Craigie are complementary volumes, ranging from methods for culturing algae, techniques for studying their subcellular components, to new techniques for exploring their development and structure as whole cells.

Contributions were requested from a number of investigators of algal systems. Many others were considered, but it was not possible to ask for their contributions, because almost everyone who was asked agreed cheerfully and some limit had to be imposed on the length of the volume. The interest of the members of the Phycological Society of America and the offers of many to help have been particularly gratifying.

Production of a multi-author effort such as this, with its 32 chapters and 46 contributors, is subject to certain causes of delay, a major one being the editor's optimism and unfamiliarity with the process of publication. I am extremely grateful for the cooperation of the contributors and for the quality of the chapters that they have produced. It is after all their volume; my job as editor was chiefly to strive for as much uniformity and clarity as possible.

My experience with the editorial committee completely belies the old adage about the ineffectiveness of a committee. The advice of the committee members was invaluable. Their suggestions of topics and contributors broadened the coverage of the volume considerably, and they generously shared in the review of the submitted manuscripts.

Grateful acknowledgment is made of the help of the many phycologists who reviewed the various chapters, and my thanks go to Claudia A. Lipschultz, Karen Applestein, and Joan HajShafi of the Smithson-

ian Institution for their daily involvement and cheerful help in preparing the manuscripts for publication.

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Contents

Introduction

The general intent of this collection of chapters is to stimulate the investigation of algae 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).

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Section I

Experimental algal systems and techniques

1: Control of morphogenesis in Micrasterias

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CONTENTS

I. Objective

Micrasterias, 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 *Micrasterias* is classified in the family of Desmidiaceae. In the system of Fritsch (1961), Desmidiaceae are considered a suborder –Desmidioidae (placoderm Desmids)–of the Conjugales. Our test object, *Micrasterias 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