

---

# Advances in Experimental Medicine and Biology

Volume 979

## **Editorial Board**

IRUN R. COHEN, *The Weizmann Institute of Science, Rehovot, Israel*

N.S. ABEL LAJTHA, *Kline Institute for Psychiatric Research, Orangeburg,  
NY, USA*

JOHN D. LAMBRIS, *University of Pennsylvania, Philadelphia, PA, USA*

RODOLFO PAOLETTI, *University of Milan, Milan, Italy*

---

Steven D. Schwartzbach  
Shigeru Shigeoka  
Editors

# Euglena: Biochemistry, Cell and Molecular Biology

 Springer

*Editors*

Steven D. Schwartzbach  
Department of Biological Sciences  
University of Memphis  
Memphis, TN, USA

Shigeru Shigeoka  
Faculty of Agriculture  
Kindai University  
Nara, Japan

ISSN 0065-2598                      ISSN 2214-8019 (electronic)  
Advances in Experimental Medicine and Biology  
ISBN 978-3-319-54908-8              ISBN 978-3-319-54910-1 (eBook)  
DOI 10.1007/978-3-319-54910-1

Library of Congress Control Number: 2017936664

© Springer International Publishing AG 2017

This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Printed on acid-free paper

This Springer imprint is published by Springer Nature  
The registered company is Springer International Publishing AG  
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

*This volume is dedicated to the trailblazers, the late Shozaburo Kitaoka and the late Jerome A. Schiff, who recognized the potential of Euglena and over the years provided training, encouragement, and guidance as we endeavored to shed light on its fascinating and novel biology.*

---

## Preface

For over 50 years, *Euglena* has been an organism of choice for addressing fundamental questions in eukaryotic biochemistry and cellular and molecular biology. *Euglena* grows rapidly in the dark to high cell densities using a diverse array of organic compounds and industrial waste streams. Rapid growth is also achieved in the light using photosynthesis as the sole source of carbon and energy for growth or using both photosynthesis and organic carbon. Grown in the dark, *Euglena* has a poorly developed plastid which upon light exposure develops into a photosynthetically competent chloroplast. Unique among photosynthetic organisms, the chloroplast is totally gratuitous to growth. Permanently white relatives of photosynthetic euglenoids are found in nature, and chloroplast loss is readily induced by a variety of treatments in the laboratory. Due to a rapid growth rate combined with simple nutritional requirements, the large number of cells required for isolation of subcellular organelles can easily be obtained in less than a week allowing both in vivo and in vitro studies of organelle biochemistry and molecular biology.

The availability of large quantities of permanently white and green *Euglena* resulted in the *Euglena* chloroplast being among the first photosynthetic organelles whose genome was physically characterized and sequenced. The undeveloped plastids in dark-grown *Euglena*, the facile induction by light exposure of the enzymatic machinery required to transform the plastid into a photosynthetically competent chloroplast, and the subcellular organelle which allows CO<sub>2</sub> to be used as the sole source of carbon and energy for growth made *Euglena* a model system for studies of organelle biogenesis. Studies of *Euglena* were instrumental in establishing the contribution of the nuclear and chloroplast genome to the development of chloroplasts. As additional information became available from studies of higher plants and other algae, it became apparent that *Euglena* was an atypical organism; it had its own way of doing things. The focus of *Euglena* research shifted from being a model organism to being an organism that could be used to elucidate novel patterns of genome organization, transcript processing, gene expression, and transport of proteins to chloroplasts providing insights into the evolutionary origin of eukaryotic cellular processes.

The diverse nature of organic carbon compounds and industrial waste streams supporting growth, the ability to grow photosynthetically, and the evolutionary relationship to the parasitic trypanosomes make *Euglena* an organism of choice for investigations of diverse and in some cases unique

biochemical pathways. *Euglena* mitochondria are the site of a classical respiratory pathway, an alternative respiratory pathway, and an anaerobic fermentation pathway producing wax esters. The photorespiratory pathway and glyoxylate pathway for ethanol assimilation are localized in most organisms to specialized organelles called microbodies, but these metabolic pathways appear to be localized to *Euglena* mitochondria. During photosynthetic and aerobic growth, *Euglena* produces a  $\beta$ -1,3-glucan, paramylum, as the major storage product, while wax esters are accumulated during mitochondrial anaerobic fermentation. A number of uses for paramylum have been found, and the *Euglena* wax ester has potential as a biofuel. *Euglena* accumulates nutraceuticals and cosmeceuticals, and its biomass has a large nutritional value. The novel nature of the biochemical pathways found in *Euglena* makes it a source of genes for genetic engineering of other organisms for production of high-value compounds.

The facile large-scale cultivation of *Euglena* on a wide array of organic compounds and industrial waste streams over a pH range of approximately 3.5–9 taken together with its metabolic diversity suggests a multitude of potential biotechnology applications. To make this potential a reality requires an in-depth understanding of the biochemistry and cellular and molecular biology of *Euglena*. Our current knowledge regarding these topics is plentiful, but it is scattered throughout the plant, protist, biochemical cell biology, and evolutionary biology literature of the past 50 years. It is over 25 years since the final volume of the four-volume compilation of *Euglena* biochemical and molecular biology research, *The Biology of Euglena*, appeared. The 14 chapters of this volume are contributed by well-known experts who in many cases played a major role in elucidating the phenomena discussed. The content is divided into three sections. The first section describes novel biochemical pathways which in some cases have an atypical subcellular localization. The next section details atypical cellular mechanisms of organelle protein import, organelle nuclear genome interdependence, and gene regulation and expression providing insights into the evolutionary origins of eukaryotic cells. The final section discusses how biotechnologists have capitalized on the novel cellular and biochemical features of *Euglena* to produce value-added products. The reader will come away from this volume with an understanding of the atypical biochemistry and cellular and molecular biology of one organism, *Euglena*, and realize the diversity of cellular processes yet to be discovered on the different branches of the tree of life.

Memphis, TN, USA  
Nara, Japan

Steven D. Schwartzbach  
Shigeru Shigeoka

---

# Contents

## Part I Biochemistry and Physiology

- 1 Evolutionary Origin of *Euglena*** ..... 3  
Bożena Zakryś, Rafał Milanowski, and Anna Karnkowska
- 2 The Mitochondrion of *Euglena gracilis***..... 19  
Verena Zimorski, Cessa Rauch, Jaap J. van Hellemond,  
Aloysius G.M. Tielens, and William F. Martin
- 3 C2 metabolism in *Euglena***..... 39  
Masami Nakazawa
- 4 Biochemistry and Physiology of Reactive Oxygen Species  
in *Euglena*** ..... 47  
Takahiro Ishikawa, Shun Tamaki, Takanori Maruta, and Shigeru  
Shigeoka
- 5 Biochemistry and Physiology of Vitamins in *Euglena*** ..... 65  
Fumio Watanabe, Kazuya Yoshimura, and Shigeru Shigeoka
- 6 Biochemistry and Physiology of Heavy Metal Resistance  
and Accumulation in *Euglena***..... 91  
Rafael Moreno-Sánchez, Sara Rodríguez-Enríquez, Ricardo  
Jasso-Chávez, Emma Saavedra, and Jorge D. García-García

## Part II Cell and Molecular Biology

- 7 *Euglena gracilis* Genome and Transcriptome: Organelles,  
Nuclear Genome Assembly Strategies and Initial Features**..... 125  
ThankGod Echezona Ebenezer, Mark Carrington, Michael Lebert,  
Steven Kelly, and Mark C. Field
- 8 *Euglena* Transcript Processing** ..... 141  
David C. McWatters and Anthony G. Russell
- 9 Photo and Nutritional Regulation of *Euglena* Organelle  
Development** ..... 159  
Steven D. Schwartzbach
- 10 Protein Targeting to the Plastid of *Euglena***..... 183  
Dion G. Durnford and Steven D. Schwartzbach

---

<b>11</b>	<b>Photomovement in <i>Euglena</i></b> .....	207
	Donat-P. Häder and Mineo Iseki	
<b>12</b>	<b>Gravitaxis in <i>Euglena</i></b> .....	237
	Donat-P. Häder and Ruth Hemmersbach	
<b>Part III Biotechnology</b>		
<b>13</b>	<b>Wax Ester Fermentation and Its Application for Biofuel Production</b> .....	269
	Hiroshi Inui, Takahiro Ishikawa, and Masahiro Tamoi	
<b>14</b>	<b>Large-Scale Cultivation of <i>Euglena</i></b> .....	285
	Kengo Suzuki	
	<b>Index</b> .....	295



---

## Contributors

**Mark Carrington** Department of Biochemistry, University of Cambridge, Cambridge, UK

**Dion G. Durnford** Department of Biology, University of New Brunswick, Fredericton, NB, Canada

**ThankGod Echezona Ebenezer** Department of Biochemistry, University of Cambridge, Cambridge, UK  
School of Life Sciences, University of Dundee, Dundee, UK

**Mark C. Field** School of Life Sciences, University of Dundee, Dundee, UK

**Jorge D. García-García** Departamento de Bioquímica, Instituto Nacional de Cardiología Ignacio Chávez, Ciudad de México, Mexico

**Donat-P. Häder** Department of Biology, Friedrich-Alexander Universität, Erlangen-Nürnberg, Möhrendorf, Germany

**Ruth Hemmersbach** Gravitational Biology, Institute of Aerospace Medicine, German Aerospace Center (DLR), Cologne, Germany

**Hiroshi Inui** Department of Nutrition, Osaka Prefecture University, Osaka, Japan

**Mineo Iseki** Faculty of Pharmaceutical Sciences, Toho University, Funabashi, Chiba, Japan

**Takahiro Ishikawa** Faculty of Life and Environmental Science, Shimane University, Matsue, Shimane, Japan

**Ricardo Jasso-Chávez** Departamento de Bioquímica, Instituto Nacional de Cardiología Ignacio Chávez, Ciudad de México, Mexico

**Anna Karnkowska** Department of Molecular Phylogenetics and Evolution, Faculty of Biology, Biological and Chemical Research Centre, University of Warsaw, Warsaw, Poland

**Steven Kelly** Department of Plant Sciences, University of Oxford, Oxford, UK

**Michael Lebert** Cell Biology Division, Department of Biology, University of Erlangen-Nuremberg, Erlangen, Germany

**William F. Martin** Institute of Molecular Evolution, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany

**Takanori Maruta** Faculty of Life and Environmental Science, Shimane University, Matsue, Shimane, Japan

**David C. McWatters** Department of Biological Sciences, University of Lethbridge, Lethbridge, AB, Canada

Alberta RNA Research and Training Institute, University of Lethbridge, Lethbridge, AB, Canada

**Rafal Milanowski** Department of Molecular Phylogenetics and Evolution, Faculty of Biology, Biological and Chemical Research Centre, University of Warsaw, Warsaw, Poland

**Rafael Moreno-Sánchez** Departamento de Bioquímica, Instituto Nacional de Cardiología Ignacio Chávez, Ciudad de México, Mexico

**Masami Nakazawa** Faculty of Life and Environmental Sciences, Osaka Prefecture University, Sakai, Osaka, Japan

**Cessa Rauch** Institute of Molecular Evolution, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany

**Sara Rodríguez-Enríquez** Departamento de Bioquímica, Instituto Nacional de Cardiología Ignacio Chávez, Ciudad de México, Mexico

**Anthony G. Russell** Department of Biological Sciences, University of Lethbridge, Lethbridge, AB, Canada

Alberta RNA Research and Training Institute, University of Lethbridge, Lethbridge, AB, Canada

**Emma Saavedra** Departamento de Bioquímica, Instituto Nacional de Cardiología Ignacio Chávez, Ciudad de México, Mexico

**Steven D. Schwartzbach** Department of Biological Sciences, University of Memphis, Memphis, TN, USA

**Shigeru Shigeoka** Faculty of Agriculture, Kindai University, Nara, Japan

**Kengo Suzuki** Department of Research and Development, euglena Co., Ltd., Tokyo, Japan

**Shun Tamaki** Faculty of Life and Environmental Science, Shimane University, Matsue, Shimane, Japan

**Masahiro Tamoi** Faculty of Agriculture, Kindai University, Nara, Japan

**Aloysius G.M. Tielens** Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Center, Rotterdam, The Netherlands  
Department of Biochemistry and Cell Biology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

---

**Jaap J. van Hellemond** Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Center, Rotterdam, The Netherlands

**Fumio Watanabe** Faculty of Agriculture, School of Agricultural, Biological and Environmental Sciences, Tottori University, Tottori, Japan

**Kazuya Yoshimura** Department of Food and Nutritional Sciences, College of Bioscience and Biotechnology, Chubu University, Kasugai, Aichi, Japan

**Bożena Zakryś** Department of Molecular Phylogenetics and Evolution, Faculty of Biology, Biological and Chemical Research Centre, University of Warsaw, Warsaw, Poland

**Verena Zimorski** Institute of Molecular Evolution, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany