Thomas J. Bach · Michel Rohmer *Editors*

Isoprenoid Synthesis in Plants and Microorganisms

New Concepts and Experimental Approaches

Springer

Isoprenoid Synthesis in Plants and Microorganisms

Thomas J. Bach • Michel Rohmer
Editors

Isoprenoid Synthesis in Plants and Microorganisms

New Concepts and Experimental Approaches

Springer

Editors
Thomas J. Bach
Département "Réseaux métaboliques"
Institut de Biologie Moléculaire des Plantes (CNRS UPR 2357)
Université de Strasbourg
Strasbourg, France

Michel Rohmer
Institut de Chimie, UMR 7177
Université de Strasbourg
Strasbourg, France

ISBN 978-1-4614-4062-8 ISBN 978-1-4614-4063-5 (eBook)
DOI 10.1007/978-1-4614-4063-5
Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2012946735

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

There is an increasing number of known metabolites that are derived from the isoprenoid pathways in plants and microorganisms, which are essential in fundamental cellular processes and in whole organisms, but also in ecological interactions. Also, their role in modern industries has spurred an intense research worldwide.

Even though research on isoprenoid synthesis and function is a rapidly developing and rather competitive field, we thought it useful to collect a number of contributions written by true and well-known experts, covering a broad spectrum of aspects. Microorganisms, plants, and even a human parasite are considered. It adds the application of modern techniques in biochemistry and structural biology, not to forget the analysis and identification of a plethora of secondary metabolites.

What aspects will be touched on in this compilation of chapters? More and more the boundaries between different categories of science such as chemistry, biochemistry, molecular and cell biology, and ecology are getting blurred, and this holds true for microbiology versus plant sciences too. In this way, attempts are underway to engineer bacteria and yeast to produce high amounts of a desired metabolite originally identified in medicinal plants of low productivity or to place regulatory and biosynthetic genes under the control of strong and tissue-specific promoters followed by transformation of (crop) plants. This extends even to biosynthesize new, "unnatural" compounds, offering engineered substrates to heterologously expressed enzymes and thus opens new avenues toward the generation of new antibiotics and pharmaceuticals. However, such approaches, including the engineering of whole pathways into yeast and bacterial cells, need to be based on a solid knowledge of biosynthetic pathways and their enzymes involved. A production *in planta* requires a precise knowledge of their intracellular distribution and possible expression in specialized tissues, aiming at enhanced production, for instance, as a consequence of environmental conditions, hormonal treatment, and exposure to biological and chemical stress.

Within the last decade or so, a major focus in the realm of isoprenoid research has been on the alternative methylerythritol phosphate (MEP) pathway, and the classical mevalonic acid (MVA) route has virtually been neglected. Thus, in the compilation of chapters, this one-sided view will be corrected by various contributions. Of course, one major aim in metabolic research is to identify rate-limiting steps in biosynthetic pathways and to

elucidate possible interactions of intermediates with enzymes, causing feedback regulation. Thus, simple overexpression of some enzymes thought of regulating pathways might not always constitute the best method. Structural biology comes into play, not only to elucidate catalytic reaction pathways but also to identify allosteric binding sites and to modify them through genetic engineering, thereby deregulating a pathway for higher productivity. In view of the structural complexity of many isoprenoid-derived vitamins and pharmaceuticals, biological production rather than chemical synthesis is the method of choice. Not so rarely, what is useful in treatment of sickness (saponins, cardenolides, etc.) in the producing organism is just to become toxic to pathogens and other enemies.

Speaking of pharmaceuticals, it is also noteworthy to mention that microbial and plant isoprenoid biosyntheses constitute excellent targets for the development of specific inhibitors and herbicides. As to this aspect, it shall not be neglected that a major human parasite, *Plasmodium falciparum*, the causative agent of malaria, is susceptible to inhibitors of the so-called MVA-independent or MEP pathway, as are a series of humanopathogenic bacteria. The importance of the coexistence of such compartmentalized pathways in plant cells is broadly discussed. Similarly, plant isoprenoid-derived compounds, like the much-studied artemisinin, are still efficient in the treatment of malaria caused by strains that have become resistant to a multitude of synthetic pharmaceuticals. Artemisinin and other drugs of high value, also flavors like geraniol and linalool, are produced by engineered yeast cells.

What about plant hormone biosynthesis and their regulatory action? It should not escape the attention of the reader that most phytohormones are entirely isoprenoids (e.g., gibberellic acids, abscisic acid, brassinosteroids, strigolactones) or contain isoprenoid-derived moieties like natural cytokinins. In signaling chains, we find implicated isoprenylated proteins, even regulating the synthesis and accumulation of secondary products like alkaloids in *Catharanthus roseus*.

Plants can apparently adapt metabolic processes (like the spatially separated MVA and MEP pathways) such that survival is possible under specific stress conditions, especially when attacked by microorganisms and herbivores. Such defense reactions must be rapid and efficient as well. Under these conditions, plants focus on the optimization of metabolic flux rates for the production of suitable phytochemicals, at the expense of general growth and development, and the whole biochemical machinery is adjusted to use all available precursor pools for defense. Interestingly enough, there is evidence for partial crossover of signaling pathways, which leads to the formation of suitably adapted groups of compounds with the highest efficiency against specific enemies. This would involve changing intracellular barriers that separate reactions and key intermediates that are not exchanged under "normal" conditions. Plants emit volatile organic compounds (VOC), many of them being isoprenoids to alert neighboring plants, which react by induction of defense reactions against microbial attack, but also use such signal to attract helper insects and nematodes feeding on herbivores. Trichomes are known to store such VOC, and it is interesting to see how their number and storage capacities depend on biosynthetic pathways and which isozymes are involved.

We do not forget isoprenoid polymers like rubber and how their synthesis is regulated. Even today, the mechanical properties of natural rubber make it absolutely essential for specific industrial products, and there is a fierce search around the world to seek for plants other than *Hevea brasiliensis* that produce rubber and related natural compounds.

Systems biology comes into play in the identification of genes that are co-expressed with known isoprenoid biosynthesis genes under certain conditions and thus helps to identify those that encode enzymes in branch-specific pathways, which opens new avenues in genetic engineering. High-throughput methods, data bank mining, and so forth all require users to ask precise questions that allow the arrangement of large sets of data (which are often of little utility) into something really useful. The same holds true with application of gene silencing techniques, which enable us to eventually identify the biological and metabolic function. Those techniques need to be accompanied by studying metabolic profiles. The combination of these techniques, carried out by well-trained specialists, also in the field of informatics, will certainly put us forward.

The chapters in this book have been arranged in such a way that there is some logical relation between them. In the first part, an overlook is given on isoprenoid biosynthesis in prokaryotic organisms and their enormous biochemical plasticity and adaptation. This then extends to structural biology, namely, the characterization of the entry enzyme into the MEP pathway. Actinomycetes are a primary source of antibiotics, and the various isoprenoid compounds produced are extensively presented. A further contribution focuses on the situation in cyanobacteria, with the focus on the connections between the MEP and pentose phosphate pathways.

The yeast *Saccharomyces cerevisiae* is the vehicle of choice for biotechnological engineering, which is extensively outlined in three chapters. One major product, meanwhile at an industrial scale, is artemisinin, an antimalarial drug. Of course, it fits well to immediately thereafter discuss its production in the plant and how this might be regulated in *Artemisia annua*. As the MEP pathway seems to be essential for the causative parasite *Plasmodium falciparum*, it follows a chapter on the action and efficiency of a MEP pathway inhibitor (fosmidomycin) in treating infected patients.

The next series of chapters is arranged in the order of increasing molecular mass of isoprenoids: semi-, mono-, sesqui-, and up to diterpenes, which leads to a broad discussion on ecological and other functions, including the structural characterization of enzymes like aristolochene synthase. In the context of "defense," the ecological function of C_{16}-terpenes, derivatives of the diterpene geranyl linalool, is discussed, which neatly prepares to talk about the newest addition to the list of phytohormones, carotenoid-derived strigolactones as "a cry to help," but resulting in "fatal attraction" of parasitic plants.

Then already speaking of phytohormones, it seems reasonable to continue with gibberellic acids (GAs) that constitute a major class of diterpenes; their biosynthesis and biological action are extensively presented. When we consider GAs as primary products, it is then necessary to discuss the regulation of pathways leading to primary and secondary products, at least sharing the synthesis of common precursors like IPP and DMAPP, here synthesized via

the MEP pathway. Many such secondary products are stored in specific morphological structures such as trichomes, which present an interesting target for genetic engineering of corresponding plants.

The synthesis of many secondary products can be induced by treatment with the (stress) phytohormone jasmonate, here in the special case of monoterpenoid indole alkaloids in *Catharanthus roseus*, for instance, the pharmacologically important anticancer drugs vinblastine and vincristine. However, there is clear evidence that prenylated proteins are implicated in the signaling chain. The next chapter discusses the role of prenylated proteins, especially their processing by prenylcysteine methylation in abscisic acid (ABA) signaling. In practice, some drought resistance of plants might be increased through affecting this process.

We then go higher in the molecular mass, discussing first polyprenols, like dolichol as cofactor in protein modification by glycosylation. If we go then to polyprenol polymers as secondary products, we arrive at rubber. In *Hevea brasiliensis*, the overall synthesis in latex seems to be regulated at the level of enzymes in the MVA pathway: HMG-CoA synthase and HMG-CoA reductase. But there are other plants that produce rubber, for instance, guayule (*Parthenium argentatum*). Such plants need to be developed into industrial crops.

We again arrive at the MVA pathway, discussing its entry enzymes, acetoacetyl-CoA thiolases, which have biosynthetic and degradative functions. In the same contribution, squalene synthase, the first enzyme committed to the sterol pathway, has been silenced, which results in growth inhibition. Now arrived at the C_{30} level of triterpenes, the biochemistry of phytosterol biochemistry is exemplified with analyzing an important enzymic step, the C4-demethylation, and thereafter of the C22 desaturase reaction in the following chapter. Squalene and squalene epoxide cyclases can be used to produce unnatural cyclic terpenes, perhaps of pharmacological importance.

While phytosterols certainly belong to primary products, triterpenoid compounds like saponins and also cardenolides can be grouped into secondary metabolites; however, their ecological (and pharmacological) importance is evident!

The last chapters focus on the model plant *Arabidopsis thaliana*, going deeply into molecular biology and biochemistry, also with the help of mutants and inhibitors. Of course, some redundancy of information is unavoidable, but various aspects are discussed from different points of view. At the end, the generation of a publicly accessible data bank on all what touches on isoprenoid pathways and regulation in *A. thaliana* is presented.

Of course, a gentle reader is not forced to follow this sequence….

Strasbourg, France — Thomas J. Bach
Strasbourg, France — Michel Rohmer

Contents

1 Isoprenoid Biosynthesis in Prokaryotic Organisms 1
Manuel Rodríguez-Concepción and Albert Boronat

2 1-Deoxy-D-Xylulose 5-Phosphate Synthase (DXS), a Crucial Enzyme for Isoprenoids Biosynthesis 17
Song Xiang, Gerlinde Usunow, Gudrun Lange, Marco Busch, and Liang Tong

3 Biosynthetic Genes and Enzymes of Isoprenoids Produced by Actinomycetes 29
Tohru Dairi

4 Interactions of Isoprenoid Pathway Enzymes and Indirect Stimulation of Isoprenoid Biosynthesis by Pentose Phosphate Cycle Substrates in *Synechocystis* PCC 6803 51
Kelly Poliquin, Francis X. Cunningham Jr., R. Raymond Gantt, and Elisabeth Gantt

5 Metabolic Engineering of Monoterpenoid Production in Yeast 65
Marc Fischer, Sophie Meyer, Maryline Oswald, Patricia Claudel, and Francis Karst

6 Metabolic Engineering of Isoprenoid Production: Reconstruction of Multistep Heterologous Pathways in Tractable Hosts 73
Jérôme Maury, Mohammad A. Asadollahi, Luca R. Formenti, Michel Schalk, and Jens Nielsen

7 Microbially Derived Semisynthetic Artemisinin 91
Christopher J. Paddon, Derek McPhee, Patrick J. Westfall, Kirsten R. Benjamin, Douglas J. Pitera, Rika Regentin, Karl Fisher, Scott Fickes, Michael D. Leavell, and Jack D. Newman

8 Artemisinin: Controlling Its Production in *Artemisia annua* 107
Pamela Weathers, Melissa Towler, Yi Wang, and Kristin K. Wobbe

9 Fosmidomycin as an Antimalarial Agent 119
Jochen Wiesner, Armin Reichenberg, Martin Hintz, Regina Ortmann, Martin Schlitzer, Serge Van Calenbergh, Steffen Borrmann, Bertrand Lell, Peter G. Kremsner, David Hutchinson, and Hassan Jomaa

10 Regulation of Isoprene and Monoterpene Emission 139
Isabel Nogués and Francesco Loreto

11 Involvement of Compartmentalization in Monoterpene and Sesquiterpene Biosynthesis in Plants 155
Michael Gutensohn, Dinesh A. Nagegowda, and Natalia Dudareva

12 Strategies for the Manipulation of Carbocations by Aristolochene Synthase 171
David J. Miller and Rudolf K. Allemann

13 Elucidating the Formation of Geranyllinalool, the Precursor of the Volatile C_{16}-Homoterpene TMTT Involved in Indirect Plant Defense 185
Marco Herde, Katrin Gärtner, Tobias Köllner, Benjamin Fode, Wilhelm Boland, Jonathan Gershenzon, Christiane Gatz, and Dorothea Tholl

14 Strigolactones: A Cry for Help Results in Fatal Attraction. Is Escape Possible? 199
Carolien Ruyter-Spira, Juan Antonio López-Ráez, Catarina Cardoso, Tatsiana Charnikhova, Radoslava Matusova, Wouter Kohlen, Muhammad Jamil, Ralph Bours, Francel Verstappen, and Harro Bouwmeester

15 Prenyldiphosphate Synthases and Gibberellin Biosynthesis 213
Chris C.N. van Schie, Michel A. Haring, and Robert C. Schuurink

16 Gibberellin Phytohormone Metabolism 233
Reuben J. Peters

17 Control of Plastidial Isoprenoid Precursor Supply: Divergent *1-Deoxy-D-Xylulose 5-Phosphate Synthase* (*DXS*) Isogenes Regulate the Allocation to Primary or Secondary Metabolism 251
Michael H. Walter, Daniela S. Floss, Heike Paetzold, Kerstin Manke, Jessica Vollrath, Wolfgang Brandt, and Dieter Strack

18 Tobacco Trichomes as a Platform for Terpenoid Biosynthesis Engineering 271
Alain Tissier, Christophe Sallaud, and Denis Rontein

19 Prenylated Proteins Are Required for Methyl-Jasmonate-Induced Monoterpenoid Indole Alkaloids Biosynthesis in *Catharanthus roseus* 285
Vincent Courdavault, Marc Clastre, Andrew John Simkin, and Nathalie Giglioli-Guivarc'h

20 The Role of Prenylcysteine Methylation and Metabolism in Abscisic Acid Signaling in *Arabidopsis thaliana* 297
Dring N. Crowell and David H. Huizinga

21 What We Do and Do Not Know About the Cellular Functions of Polyisoprenoids 307
Liliana Surmacz and Ewa Swiezewska

22 Regulation of 3-Hydroxy-3-Methylglutaryl-CoA Synthase and 3-Hydroxy-3-Methylglutaryl-CoA Reductase and Rubber Biosynthesis of *Hevea brasiliensis* (B.H.K.) Mull. Arg 315
Pluang Suwanmanee, Nualpun Sirinupong, and Wallie Suvachittanont

23 Development of Crops to Produce Industrially Useful Natural Rubber 329
Maureen Whalen, Colleen McMahan, and David Shintani

24 Occurrence of Two Acetoacetyl-Coenzyme A Thiolases with Distinct Expression Patterns and Subcellular Localization in Tobacco 347
Laurent Wentzinger, Esther Gerber, Thomas J. Bach, and Marie-Andrée Hartmann

25 The Sterol C4-Demethylation in Higher Plants 367
Alain Rahier, Sylvain Darnet, Florence Bouvier, and Bilal Camara

26 Sterol C22-Desaturase and Its Biological Roles 381
Daisaku Ohta and Masaharu Mizutani

27 Enzymatic Synthesis of Unnatural Cyclic Triterpenes 393
Ikuro Abe

28 Saponin Synthesis and Function 405
Sam T. Mugford and Anne Osbourn

29 Cardenolide Aglycone Formation in *Digitalis* 425
Wolfgang Kreis and Frieder Müller-Uri

30 Biosynthesis of Isoprenoid Precursors in Arabidopsis 439
Manuel Rodríguez-Concepción, Narciso Campos, Albert Ferrer, and Albert Boronat

31 Understanding the Mechanisms that Modulate the MEP Pathway in Higher Plants 457
Patricia León and Elizabeth Cordoba

32 Functional Analysis of HMG-CoA Reductase and Oxidosqualene Cyclases in *Arabidopsis* 465
Toshiya Muranaka and Kiyoshi Ohyama

33 Systems Understanding of Isoprenoid Pathway Regulation in *Arabidopsis* 475
Eva Vranová

Index 493

Contributors

Ikuro Abe Graduate School of Pharmaceutical Sciences, The University of Tokyo, Bunkyo-ku, Tokyo, Japan

Rudolf K. Allemann School of Chemistry, Cardiff University, Cardiff, UK

Mohammad A. Asadollahi Novo Nordisk Foundation Center for Biosustainability-Technical University of Denmark, Fremtidavej 3, Denmark

Thomas J. Bach Département "Réseaux métaboliques", Institut de Biologie Moléculaire des Plantes (CNRS UPR 2357), Université de Strasbourg, Strasbourg, France

Kirsten R. Benjamin Amyris, Emeryville, CA, USA

Wilhelm Boland Department of Bioorganic Chemistry, Max-Planck-Institute for Chemical Ecology, Jena, Germany

Albert Boronat Department of Molecular Genetics, Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Barcelona, Spain

Department of Biochemistry and Molecular Biology, University of Barcelona, Barcelona, Spain

Steffen Borrmann Justus-Liebig-Universität Gießen, Institut für Klinische Immunologie und Transfusionsmedizin, Gießen, Germany

Ralph Bours Laboratory of Plant Physiology, Wageningen University, Wageningen, The Netherlands

Florence Bouvier Institut de Biologie Moléculaire des Plantes, CNRS, Strasbourg cedex, France

Harro Bouwmeester Laboratory of Plant Physiology, Wageningen University, Wageningen, The Netherlands

Wolfgang Brandt Abteilung Natur- und Wirkstoffchemie, Leibniz-Institut für Pflanzenbiochemie, Halle (Saale), Germany

Marco Busch Bayer CropScience GmbH, Industriepark Höchst, Frankfurt am Main, Germany

Serge Van Calenbergh Justus-Liebig-Universität Gießen, Institut für Klinische Immunologie und Transfusionsmedizin, Gießen, Germany

Bilal Camara Institut de Biologie Moléculaire des Plantes, CNRS, Strasbourg cedex, France

Narciso Campos Department of Molecular Genetics, Centre for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-UB, Barcelona, Spain

Department of Biochemistry and Molecular Biology, University of Barcelona, Barcelona, Spain

Catarina Cardoso Cropdesign – BASF Plant Sciences, Zwijnaarde, Belgium

Tatsiana Charnikhova Laboratory of Plant Physiology, Wageningen University, Wageningen, The Netherlands

Marc Clastre Biomolécules et Biotechnologies végétales, Université François-Rabelais de Tours Sciences et Techniques, Tours, France

Patricia Claudel UMR SVQV, University of Strasbourg, INRA, Colmar, France

Elizabeth Cordoba Departamento de Biología Molecular, Instituto de Biotecnología Universidad Autónoma de México, Morelos, Mexico

Vincent Courdavault Biomolécules et Biotechnologies végétales, Université François-Rabelais de Tours Sciences et Techniques, Tours, France

Dring N. Crowell Passed away on June 30, 2012

Francis X. Cunningham Jr. Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD, USA

Tohru Dairi Graduate School of Engineering, Hokkaido University, Sapporo, Hokkaido, Japan

Sylvain Darnet Institut de Biologie Moléculaire des Plantes, CNRS, Strasbourg cedex, France

Natalia Dudareva Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN, USA

Albert Ferrer Faculty of Pharmacy, Department of Molecular Genetics, Centre for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-UB, Barcelona, Spain

Department of Biochemistry and Molecular Biology, University of Barcelona, Barcelona, Spain

Scott Fickes Amyris Biotechnologies, Emeryville, CA, USA

Marc Fischer UMR SVQV, University of Strasbourg, INRA, Colmar, France

Karl Fisher Amyris Biotechnologies, Emeryville, CA, USA

Daniela S. Floss Abteilung Sekundärstoffwechsel, Leibniz-Institut für Pflanzenbiochemie, Halle (Saale), Germany

Benjamin Fode Albrecht-von Haller-Institute for Plant Sciences, Georg-August-University Göttingen, Göttingen D-37073, Germany

Luca R. Formenti National Food Institute, Kgs Lyngby, Denmark

Elisabeth Gantt Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD, USA

R. Raymond Gantt Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD, USA

Katrin Gärtner Albrecht-von Haller-Institute for Plant Sciences, Georg-August-University Göttingen, Göttingen D-37073, Germany

Christiane Gatz Albrecht-von Haller-Institute for Plant Sciences, Georg-August-University Göttingen, Göttingen D-37073, Germany

Esther Gerber Department of Genetic Engineering, Deinove Company, Paris, France

Jonathan Gershenzon Department of Biochemistry, Max Planck Institute for Chemical Ecology, Jena, Germany

Nathalie Giglioli-Guivarc'h Biomolécules et Biotechnologies végétales, Université François-Rabelais de Tours Sciences et Techniques, Tours, France

Michael Gutensohn Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN, USA

Michel A. Haring Department of Plant Physiology, Swammerdam Institute for Life Sciences, Amsterdam, The Netherlands

Marie-Andrée Hartmann Département "Réseaux métaboliques", Institut de Biologie Moléculaire des Plantes (CNRS UPR 2357), Université de Strasbourg, Strasbourg, France

Marco Herde Albrecht-von Haller-Institute for Plant Sciences, Georg-August-University Göttingen, GöttingenD-37073, Germany

Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI, USA

Martin Hintz Justus-Liebig-Universität Gießen, Institut für Klinische Immunologie und Transfusionsmedizin, Gießen, Germany

David H. Huizinga Dow AgroSciences LLC, Indianapolis, IN, USA

David Hutchinson Justus-Liebig-Universität Gießen, Institut für Klinische Immunologie und Transfusionsmedizin, Gießen, Germany

Muhammad Jamil Laboratory of Plant Physiology, Wageningen University, Wageningen, The Netherlands

Hassan Jomaa Justus-Liebig-Universität Gießen, Institut für Klinische Immunologie und Transfusionsmedizin, Gießen, Germany

Francis Karst UMR SVQV, University of Strasbourg, INRA, Colmar, France

Wouter Kohlen Department of Plant Physiology, Wageningen University, Wageningen, The Netherlands

Tobias Köllner Department of Biochemistry, Max-Planck-Institute for Chemical Ecology, Jena, Germany

Wolfgang Kreis Lehrstuhl für Pharmazeutische Biologie, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany

ECROPS, Erlangen Center of Plant Science, Erlangen, Germany

Peter G. Kremsner Justus-Liebig-Universität Gießen, Institut für Klinische Immunologie und Transfusionsmedizin, Gießen, Germany

Gudrun Lange Bayer CropScience GmbH, Industriepark Höchst, Frankfurt am Main, Germany

Michael D. Leavell Amyris Biotechnologies, Emeryville, CA, USA

Bertrand Lell Justus-Liebig-Universität Gießen, Institut für Klinische Immunologie und Transfusionsmedizin, Gießen, Germany

Patricia León Departamento de Biología Molecular, Instituto de Biotecnología Universidad Autónoma de México, Morelos, Mexico

Juan Antonio López-Ráez Soil Microbiology and Symbiotic Systems, Estacion Experimental del Zaidin (CSIC), Granada, Spain

Francesco Loreto CNR – Istituto per la Protezione delle Piante, Sesto Fiorentino (Firenze), Italy

Kerstin Manke Abteilung Sekundärstoffwechsel, Leibniz-Institut für Pflanzenbiochemie, Halle (Saale), Germany

Radoslava Matusova Institute of Plant Genetics and Biotechnology, Slovak Academy of Sciences, Nitra, Slovakia

Jérôme Maury Center for Microbial Biotechnology, DTU-Biosys, Kgs Lyngby, Denmark

Colleen McMahan Crop Improvement and Utilization Unit, Western Regional Research Center, ARS-USDA, Albany, CA, USA

Derek McPhee Amyris Biotechnologies, Emeryville, CA, USA

Sophie Meyer UMR SVQV, University of Strasbourg, INRA, Colmar, France

David J. Miller School of Chemistry, Cardiff University, Cardiff, UK

Masaharu Mizutani Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Sakai, Japan

Sam T. Mugford Department of Metabolic Biology, John Innes Centre, Norwich, UK

Frieder Müller-Uri Lehrstuhl für Pharmazeutische Biologie, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany

ECROPS, Erlangen Center of Plant Science, Erlangen, Germany

Toshiya Muranaka Department of Biotechnology, Graduate School of Engineering, Osaka University, Osaka, Japan

Dinesh A. Nagegowda Central Institute of Medicinal and Aromatic Plants India, Plant Biotechnology Division, Lucknow, India

Jack D. Newman Amyris Biotechnologies, Emeryville, CA, USA

Jens Nielsen Department of Chemical and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden

Isabel Nogués CNR – Istituto di Biologia Agroambientale e Forestale, Monterotondo Scalo (Roma), Italy

Daisaku Ohta Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Sakai, Japan

Kiyoshi Ohyama Department of Chemistry and Materials Science, Tokyo Institute of Technology, Meguro, Tokyo, Japan

Regina Ortmann Justus-Liebig-Universität Gießen, Institut für Klinische Immunologie und Transfusionsmedizin, Gießen, Germany

Anne Osbourn Department of Metabolic Biology, John Innes Centre, Norwich, UK

Maryline Oswald UMR SVQV, University of Strasbourg, INRA, Colmar, France

Christopher J. Paddon Amyris Biotechnologies, Emeryville, CA, USA

Heike Paetzold Abteilung Sekundärstoffwechsel, Leibniz-Institut für Pflanzenbiochemie, Halle (Saale), Germany

Reuben J. Peters Department of Biochemistry, Biophysics, and Molecular Biology, Iowa State University, Ames, IA, USA

Douglas J. Pitera Amyris Biotechnologies, Emeryville, CA, USA

Kelly Poliquin Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD, USA

Alain Rahier Institut de Biologie Moléculaire des Plantes, CNRS, Strasbourg cedex, France

Armin Reichenberg Justus-Liebig-Universität Gießen, Institut für Klinische Immunologie und Transfusionsmedizin, Gießen, Germany

Manuel Rodríguez-Concepción Department of Molecular Genetics, Centre for Research in Agricultural Genomics (CRAG) CSIC-IRTA-UAB-UB, Barcelona, Spain

Denis Rontein AnaScan, Gréoux les Bains, France

Carolien Ruyter-Spira Laboratory of Plant Physiology, Wageningen University, Wageningen, The Netherlands

Christophe Sallaud Functional and Applied Cereal Group (FAC), Clermont-Ferrand Cedex, France

Michel Schalk Biotechnology Department, Firmenich SA, Geneva, Switzerland

Chris C.N. van Schie Division of Biological Sciences, University of California, San Diego, La Jolla, CA, USA

David Shintani Department of Biochemistry, University of Nevada, Reno, NV, USA

Martin Schlitzer Justus-Liebig-Universität Gießen, Institut für Klinische Immunologie und Transfusionsmedizin, Gießen, Germany

Robert C. Schuurink Department of Plant Physiology, Swammerdam Institute for Life Sciences, Amsterdam, The Netherlands

Andrew John Simkin Faculté de pharmacie Biomolécules et Biotechnologies végétales, Université François-Rabelais de Tours Sciences et Techniques, Tours, France

Nualpun Sirinupong Biochemistry and Molecular Biology, Wayne State University School of Medicine, Detroit, MI, USA

Dieter Strack Abteilung Sekundärstoffwechsel, Leibniz-Institut für Pflanzenbiochemie, Halle (Saale), Germany

Liliana Surmacz Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

Wallie Suvachittanont Faculty of Science Biochemistry Department, Prince of Songkla University, Hat Yai, Songkhla, Thailand

Pluang Suwanmanee Faculty of Science Biology Department, Thaksin University, Songkhla, Thailand

Ewa Swiezewska Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

Dorothea Tholl Department of Biological Sciences, Virginia Tech, Blacksburg, VA, USA

Alain Tissier Leibniz Institute of Plant Biochemistry, Halle (Saale), Germany

Liang Tong Department of Biological Sciences, Columbia University, New York, NY, USA

Melissa Towler Biology and Biotechnology Department, Worcester Polytechnic Institute, Worcester, MA, USA

Gerlinde Usunow Bayer CropScience GmbH, Industriepark Höchst, Frankfurt am Main, Germany

Francel Verstappen Laboratory of Plant Physiology, Wageningen University, Wageningen, The Netherlands

Jessica Vollrath Abteilung Sekundärstoffwechsel, Leibniz-Institut für Pflanzenbiochemie, Halle (Saale), Germany

Eva Vranová Department of Plant Biotechnology, Institute of Plant Sciences, Zurich, Switzerland

Michael H. Walter Abteilung Sekundärstoffwechsel, Leibniz-Institut für Pflanzenbiochemie, Halle (Saale), Germany

Yi Wang Biology and Biotechnology Department, Worcester Polytechnic Institute, Worcester, MA, USA

Pamela Weathers Biology and Biotechnology Department, Worcester Polytechnic Institute, Worcester, MA, USA

Laurent Wentzinger Actelion Pharmaceuticals Ltd, Allschwil, Switzerland

Patrick J. Westfall Amyris Biotechnologies, Emeryville, CA, USA

Maureen Whalen Crop Improvement and Utilization Unit, Western Regional Research Center, ARS-USDA, Albany, CA, USA

Jochen Wiesner Justus-Liebig-Universität Gießen, Institut für Klinische Immunologie und Transfusionsmedizin, Gießen, Germany

Kristin K. Wobbe Chemistry and Biochemistry Department, Worcester Polytechnic Institute, Worcester, MA, USA

Song Xiang Department of Biological Sciences, Columbia University, New York, NY, USA

Isoprenoid Biosynthesis in Prokaryotic Organisms

1

Manuel Rodríguez-Concepción and Albert Boronat

Abstract

Isoprenoids are ubiquitous compounds found in all living organisms. In spite of their remarkable diversity of structures and functions, all isoprenoids derive from a basic five-carbon precursor unit, isopentenyl diphosphate (IPP), and its isomer dimethylallyl diphosphate (DMAPP). Addition of IPP units to DMAPP, catalyzed by prenyltransferases, results in the synthesis of prenyl diphosphates of increasing length which are the starting points of downstream pathways leading to the synthesis of the different isoprenoid end products. For many years, it was accepted that IPP was synthesized from acetyl-CoA through the well-known mevalonate (MVA) pathway. However, an alternative MVA-independent pathway for the biosynthesis of IPP and DMAPP was identified a few years ago in bacteria, algae, and plants. This novel pathway, currently known as the methylerythritol 4-phosphate (MEP) pathway, is widely distributed in nature and is present in most eubacteria. Here, we describe the biological relevance of the main isoprenoid compounds found in prokaryotic organisms and the metabolic origin of the IPP and DMAPP used for their synthesis, with a particular emphasis on those isoprenoids present in the

M. Rodríguez-Concepción (✉)
Department of Molecular Genetics,
Centre for Research in Agricultural Genomics (CRAG)
CSIC-IRTA-UAB-UB, Campus UAB Bellaterra,
Barcelona E-08193, Spain
e-mail: manuel.rodriguez@cragenomica.es

A. Boronat
Department of Molecular Genetics,
Centre for Research in Agricultural Genomics (CRAG)
CSIC-IRTA-UAB-UB, Campus UAB Bellaterra,
Barcelona E-08193, Spain

Department of Biochemistry and Molecular Biology,
Faculty of Biology, University of Barcelona,
Barcelona E-08028, Spain

T.J. Bach and M. Rohmer (eds.), *Isoprenoid Synthesis in Plants and Microorganisms: New Concepts and Experimental Approaches*, DOI 10.1007/978-1-4614-4063-5_1,

model bacteria *Escherichia coli*. Since the MEP pathway is essential in most pathogenic bacteria but is absent in animals (including humans), which synthesize isoprenoids through the MVA pathway, we also describe the recent and increasing interest of the MEP pathway enzymes as targets for the development of new antibiotics.

Keywords
Archaebacteria • Dimethylallyl diphosphate • *Escherichia coli* • Eubacteria • Isoprenoid • Isopentenyl diphosphate • Terpenoid • Mevalonate • Methylerythritol 4-phosphate

1.1 Introduction

Isoprenoids (also known as terpenoids) are ubiquitous compounds found in all living organisms. In spite of their remarkable diversity of structures and functions, all isoprenoids derive from a basic five-carbon precursor unit, isopentenyl diphosphate (IPP), and its isomer dimethylallyl diphosphate (DMAPP) (Fig. 1.1). Addition of IPP units to DMAPP, catalyzed by prenyltransferases, results in the synthesis of prenyl diphosphates of increasing length (e.g., farnesyl diphosphate and geranylgeranyl diphosphate) which are the starting points of downstream pathways leading to the synthesis of the different isoprenoid end products (Fig. 1.2). For many years, it was accepted that IPP was synthesized from acetyl-CoA through the well-known mevalonate (MVA) pathway. However, an alternative MVA-independent pathway for the biosynthesis of IPP and DMAPP was identified a few years ago in bacteria, algae, and plants (Rohmer 1999; Lichtenthaler 1999). This novel pathway, currently known as the methylerythritol 4-phosphate (MEP) pathway (cf. Phillips et al. 2008), is widely distributed in nature and is present in most eubacteria, apicomplexan protozoa (like the malaria parasite *Plasmodium falciparum*), green algae, and higher plants. For a detailed description of the discovery and elucidation of the MEP pathway, we refer to other reviews on this topic (Rodríguez-Concepción and Boronat 2002; Kuzuyama and Seto 2003; Eisenreich et al. 2004; Rohmer 2008).

Here, we describe the biological relevance of the main isoprenoid compounds found in prokaryotic organisms and the metabolic origin of the isoprene units used for their synthesis, with a particular emphasis on those isoprenoids present in the model bacteria *Escherichia coli*. Since the MEP pathway is essential in most pathogenic bacteria but is absent in animals (including humans), which synthesize isoprenoids through the MVA pathway, we also describe the recent and increasing interest of the MEP pathway enzymes as targets for the development of new antibiotics.

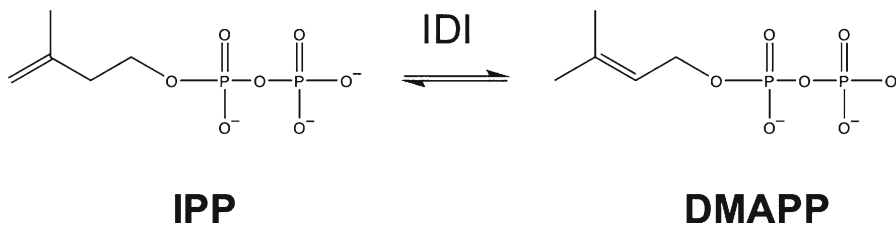

Fig. 1.1 Chemical structure of isopentenyl diphosphate (*IPP*) and dimethylallyl diphosphate (*DMAPP*) and their interconversion by the enzyme isopentenyl diphosphate isomerase (*IDI*)

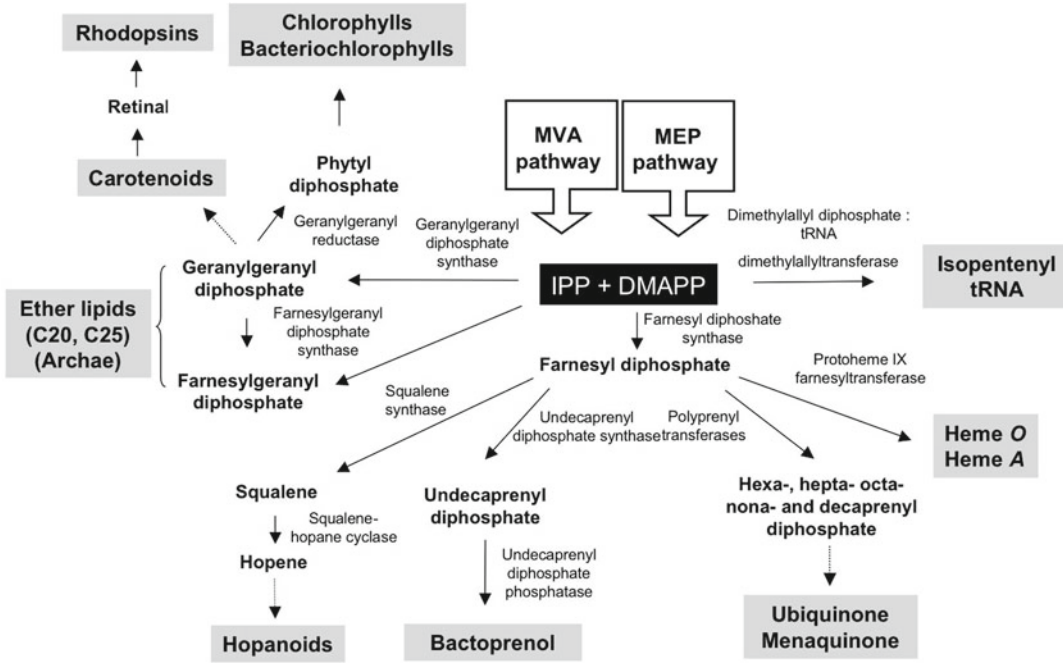

Fig. 1.2 Schematic representation of isoprenoid biosynthesis in prokaryotic organisms

1.2 Biological Relevance of Isoprenoids in the Prokaryotic World

Many isoprenoids play essential roles in a variety of processes that are vital for the growth and survival of prokaryotic organisms. They include, among others, cell wall and membrane biosynthesis, electron transport, and conversion of light into chemical energy. Also, many microorganisms produce isoprenoid secondary metabolites of biotechnological relevance. A number of review articles have recently covered the different strategies and outcomes of the metabolic engineering of isoprenoids in bacteria (Klein-Marcuschamer et al. 2007; Ajikumar et al. 2008; Kirby and Keasling 2008, 2009; Misawa 2011).

1.2.1 Cell Wall Biosynthesis: Bactoprenol

The bacterial cell wall is a rigid structure that surrounds the cytoplasmic membrane and plays an essential role in maintaining the cell shape and preventing the deleterious effect of the internal osmotic pressure. It also serves as a scaffold for anchoring other cell envelope components such as proteins and teichoic acids. Peptidoglycan is the major component of bacterial cell walls and consists in long glycan chains made up of alternating N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) residues cross-linked together by bridges made of amino acids and amino acid derivatives (Vollmer et al. 2008). The biosynthesis of peptidoglycan is a complex process that involves about 20 reactions that take place in the cytoplasm and the inner and outer sides of the cytoplasmic membrane. A key component in the synthesis of peptidoglycan is undecaprenyl phosphate, also referred to as bactoprenol (Fig. 1.3). This isoprenoid compound is needed for the synthesis and transport of hydrophilic GlcNAc-MurNAc-peptide monomeric units outside the cytoplasmic membrane, the site for peptidoglycan polymerization. Undecaprenyl phosphate derives from undecaprenyl diphosphate, a prenyl diphosphate synthesized from farnesyl diphosphate (FPP) (Fig. 1.2).

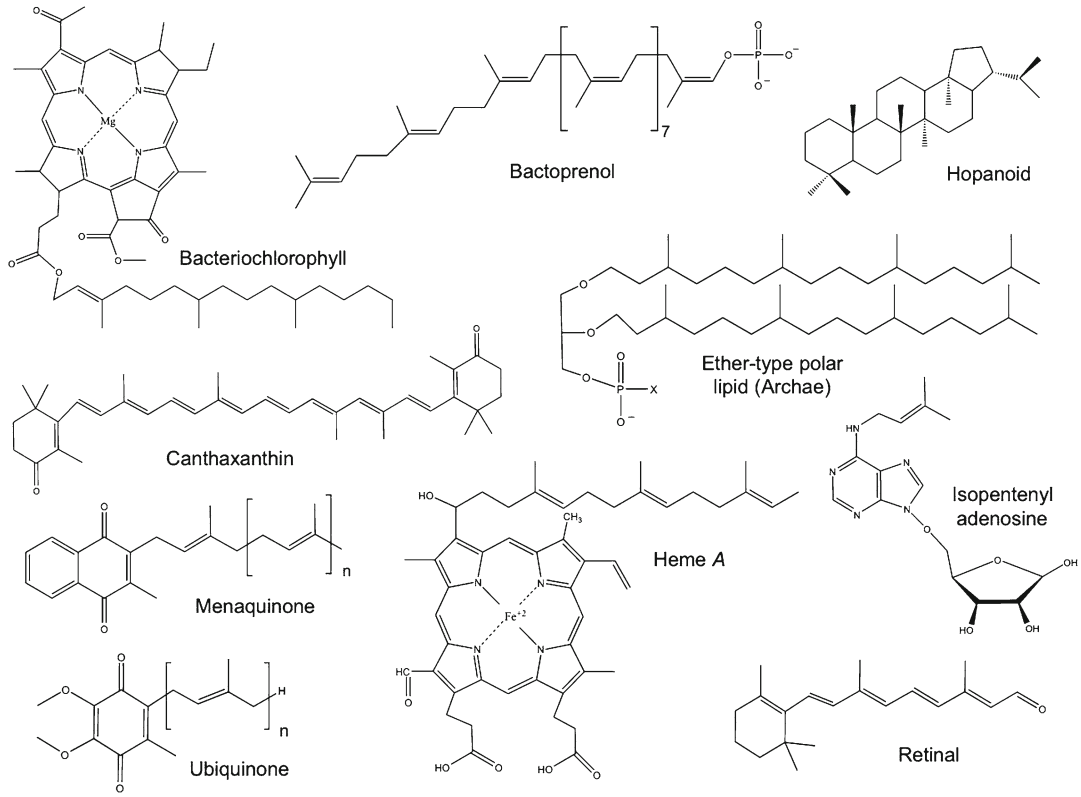

Fig. 1.3 Representative isoprenoids found in prokaryotic organisms

The enzymes involved in bactoprenol biosynthesis are undecaprenyl diphosphate synthase, which adds eight molecules of IPP (in *cis* form) onto FPP and undecaprenyl diphosphate phosphatase, which removes a phosphate group. The genes encoding these enzymes in *E. coli* (*ispU* and *bacA*, respectively) are indicated in Table 1.1. The biosynthesis of bactoprenol and its role in peptidoglycan biosynthesis have recently been reviewed by Bouhss et al. (2008).

1.2.2 Membrane Lipids: Hopanoids and Archaeal Ether-Type Lipids

Biological membranes are composed of lipids and proteins. The main membrane lipids are glycerolipids, composed of a polar head and two hydrophobic alkane groups that are mobile in the interior of the membrane. To regulate membrane fluidity, eukaryotic cells employ sterols (e.g., cholesterol). Since it is known that most prokaryotes lack sterols, the search for alternative molecules playing an equivalent role in prokaryotes has been an ongoing issue during the last decades. It is now well established that many bacteria contain hopanoids, pentacyclic triterpenoid compounds with a structure similar to that of sterols (Fig. 1.3). Hopanoids have been detected in about 30% of all bacteria investigated, including a wide range of Gram-negative and Gram-positive bacteria. Although hopanoids predominantly occur in aerobic bacteria, they have also been found in some facultative anaerobic bacteria. Hopanoids have not been detected in archaebacteria, which produce a particular membrane lipid of isoprenoid nature (see below). The hopanoid skeleton is formed from squalene by the action of squalene-hopene cyclase (Fig. 1.2). Hopanoid chemistry, biosynthesis, function, and distribution have been reviewed by Kannenberg and Poralla (1999).

Table 1.1 Genes encoding isoprenoid biosynthetic enzymes in *E. coli*[a]

Enzyme	Gene	Alternative gene symbol	Minute	Left end	Right end	Cotranscribed with
1-Deoxy-D-xylulose 5-phosphate synthase	*dxs*	*yajP*	9.43	437,539	439,401	*ispA*
1-Deoxy-D-xylulose 5-phosphate reductoisomerase	*dxr*	*ispC, yaeM*	4.17	193,521	194,717	–
2-*C*-methyl-D-erythritol 4-phosphate cytidylyltransferase	*ispD*	*ygbP*	61.85	2,869,802	2,870,512	*ispF*
4-(Cytidine 5′-diphospho)-2-*C*-methyl-D-erythritol kinase	*ispE*	*ipk, ychB*	27.18	1,261,249	1,262,100	–
2*C*-Methyl-D-erythritol 2,4-cyclodiphosphate synthase	*ispF*	*ygbB*	61.84	2,869,323	2,869,802	*ispD*
4-Hydroxy-3-methylbut-2-enyl diphosphate synthase	*ispG*	*gcpE*	56.87	2,638,708	2,639,826	–
4-Hydroxy-3-methylbut-2-enyl diphosphate reductase	*ispH*	*lytB, yaaE*	0.57	26,277	27,227	–
Isopentenyl diphosphate isomerase	*idi*	*ygfV*	65.33	3,031,087	3,031,635	–
Farnesyl diphosphate synthase	*ispA*	–	9.47	439,426	440,325	*dxs*
Octaprenyl-diphosphate synthase	*ispB*	*cel, yhbD*	71.81	3,331,732	3,332,703	–
Undecaprenyl diphosphate synthase	*ispU*	*uppS, rth, yaeS*	4.20	194,903	195,664	–
Undecaprenyl diphosphate phosphatase	*bacA*	*uppP*	69.0	3,201,332	3,202,153	–
Dimethylallyl diphosphate: tRNA dimethylallyltransferase	*miaA*	*trpX*	94.78	4397275	4,398,225	–
Protoheme IX farnesyltransferase	*cyoE*	–	9.61	446,039	446,929	–

[a]EcoGene Website/EcoWeb (http://ecogene.org/index.php)

Archaebacteria are unicellular microorganisms which evolved separately from eubacteria and eukaryotes and that are often found inhabiting extreme environments such as hot springs and salt lakes. They are similar to eubacteria in being prokaryotes (and thus lacking a nucleus) but differ from them in ribosomal structure, the presence of introns (in some species) and in membrane structure and composition. One of the most remarkable features of archaebacteria is the presence of ether-type lipids in their membranes containing hydrocarbon chains of isoprenoid nature (usually C20 phytanyl and C25 farnesylgeranyl groups) linked to *sn*-glycerol 1-phosphate (Fig. 1.3). The C20 prenyl chains may derive from geranylgeranyl diphosphate (GGPP) synthesized from IPP and DMAPP by the action of GGPP synthase (Fig. 1.2). C25 farnesylgeranyl groups are synthesized by farnesylgeranyl diphosphate synthase (Fig. 1.2) either by the consecutive condensation of IPP molecules to DMAPP or to prenyl diphosphates, like GGPP (Boucher et al. 2004). Archaeal polyprenyl diphosphate synthases can also synthesize products of shorter chain lengths. The biosynthesis of ether-type lipids in archaebacteria has recently been reviewed by Koga and Morii (2007).

1.2.3 Electron Transfer: Ubiquinone, Menaquinone, and Heme A

Ubiquinone and menaquinone are lipid-soluble molecules playing important roles in respiration. They are involved in electron transport processes between membrane-bound protein complexes in the respiratory electron-transport chain. Both compounds contain a quinone moiety linked to an isoprenoid side chain (Fig. 1.3). Ubiquinone has a benzoquinone group that is linked to an isoprenoid chain of different length (6–10 isoprene units) depending on the organism. In *E. coli* the isoprenoid side chain of ubiquinone contains 8 isoprene units. Menaquinone has a naphthoquinone ring linked to an isoprene tail, which also contains 8 isoprene units in *E. coli*. Facultative anaerobes, like *E. coli*, use ubiquinone when growing under aerobic conditions and menaquinone under anaerobic conditions. By contrast, many Gram-positive aerobes, such as *Bacillus subtilis*, contain only menaquinone. In *E. coli*, the side chain of ubiquinone and menaquinone is synthesized from FPP by octaprenyl-diphosphate synthase (Fig. 1.2), which is encoded by the essential gene *ispB* (Table 1.1) (Meganathan 2001; Kawamukai 2002).

Cytochromes are membrane-bound proteins also involved in electron-transport processes. Cytochromes contain a prosthetic group, called heme, composed by a heterocyclic porphyrin and a metal ion (usually iron) in a central position. Heme *A* (Fig. 1.3), which is found in cytochrome c and cytochrome c oxidase, is characterized by containing a hydroxyfarnesyl group. *E. coli* has no cytochrome *c* and no equivalent to the mitochondrial complex III (bc_1 complex) or complex IV (cytochrome *c* oxidase). Instead, two enzymes in the *E. coli* cytoplasmic membrane, the cytochromes *bo* and *d*, oxidize ubiquinol and directly reduce molecular oxygen to water. Cytochrome *bo* contains heme O, which only differs from heme A by having a methyl group instead of a formyl group. Heme A is derived from heme B (protoheme IX) with heme O as a probable intermediate (Mogi et al. 1994). The transfer of the farnesyl group from FPP to heme B to form heme O is catalyzed by a farnesyltransferase encoded by the *cyoE* gene in *E. coli* (Table 1.1) (Mogi et al. 1994).

1.2.4 Protein Synthesis: Isopentenyl tRNA

Transfer RNA (tRNA) molecules usually contain modified nucleotides. In almost all the tRNAs reading codons beginning with U, the adenosine at position 37 (adjacent to the 3′ position of the anticodon) is modified to N(6)-(Δ 2)-isopentenyl adenosine (Fig. 1.3) by the action of a tRNA isopentenyltransferase, encoded by the *miaA* gene in *E. coli* (Table 1.1). Homologs of the *E. coli miaA* gene have been detected in other microorganisms. Isopentenyl adenosine increases the efficiency of translation of the modified tRNAs and makes them less sensitive to codon context. Although *miaA* is not essential in *E. coli*, mutants defective

in this gene show increased rates of spontaneous mutations and altered read through and suppression of nonsense codons (Persson et al. 1994).

1.2.5 Phototrophy: Chlorophylls, Bacteriochlorophylls, Rhodopsins, and Carotenoids

Microorganisms can use two mechanisms for the conversion of light into chemical energy. One of them is dependent on photochemical reaction centers that contain chlorophylls or bacteriochlorophylls. The other mechanism employs proteorhodopsins and bacteriorhodopsins, retinal-binding membrane proteins belonging to the rhodopsin family (Bryant and Frigaard 2006).

Like plant chlorophylls, bacterial chlorophylls also contain a long isoprenoid chain (usually phytol) that contributes to their localization in the photosynthetic membranes (Fig. 1.3). The last stage in the biosynthesis of bacterial chlorophylls consists in the addition of the prenyl chain to the corresponding chlorophyllides (Gomez Maqueo Chew and Bryant 2007). It has been proposed that phytol is formed after the addition of a geranylgeraniol group which is later sequentially saturated by geranylgeranyl reductase. However, some evidences suggest that geranylgeranyl reductase can saturate GGPP prior to the transfer of the phytol tail (Fig. 1.2). Although the phytol group is present in most bacterial chlorophylls, other isoprenoids (like farnesyl, geranylgeranyl, and 2,6-phytadienyl groups) have also been reported (Gomez Maqueo Chew and Bryant 2007).

Proteorhodopsins are retinal-binding membrane proteins belonging to the rhodopsin family. Prokaryotic members of this family include energy-conserving transmembrane proton pumps, transmembrane chloride pumps, and photosensors (sensory rhodopsins) (Fuhrman et al. 2008). Originally discovered in archaebacteria, it is currently estimated that a large proportion of marine bacteria contain proteorhodopsin. In Archaea and most bacteria, retinal (Fig. 1.3) is synthesized by the oxidative cleavage of β-carotene. However, recent reports on the characterization of apocarotenoid oxygenases from cyanobacteria have shown that retinal can also be produced by cleavage of some apocarotenoids. At present, the substrate(s) used for retinal production in some bacteria remains an open question (Maresca et al. 2008).

Some photosynthetic bacteria also contain carotenoids, which function primarily as photoprotective pigments but that can also participate in the light harvesting process. Like in plants, bacterial carotenoids are also synthesized from GGPP (Fig. 1.2). Bacterial carotenoid diversity and the biochemical aspects related with their biosynthesis have recently been reviewed by Maresca et al. (2008).

1.2.6 Secondary Metabolism

Prokaryotic organisms produce isoprenoid compounds and derivatives that can be included in the large and diverse family of secondary metabolites. For instance, many nonphotosynthetic bacteria can synthesize carotenoids, some of them of industrial interest (e.g., canthaxanthin and astaxanthin). Since a considerable number of microbial (and plant) carotenoid biosynthetic genes have been cloned over the past few years, it is now possible to engineer carotenoid biosynthesis in noncarotenogenic bacteria like *E. coli*. Excellent reviews describing the biochemistry and the biotechnology of carotenoids in microorganisms are available (Lee and Schmidt-Dannert 2002; Das et al. 2007; Maresca et al. 2008). Another example of prokaryotic secondary metabolites is given by the isoprenoid antibiotics produced by many actinomycete strains (Dairi 2005; Dairi, this volume).

1.3 Origin of Isoprenoid Precursors in Prokaryotic Organisms

For many years, it was accepted that IPP was synthesized through the MVA pathway in all organisms, including microorganisms. However, as indicated above, an alternative pathway for the biosynthesis of IPP (and DMAPP) was identified a few years ago in bacteria, algae, and plants. This novel pathway, known as the MEP pathway,

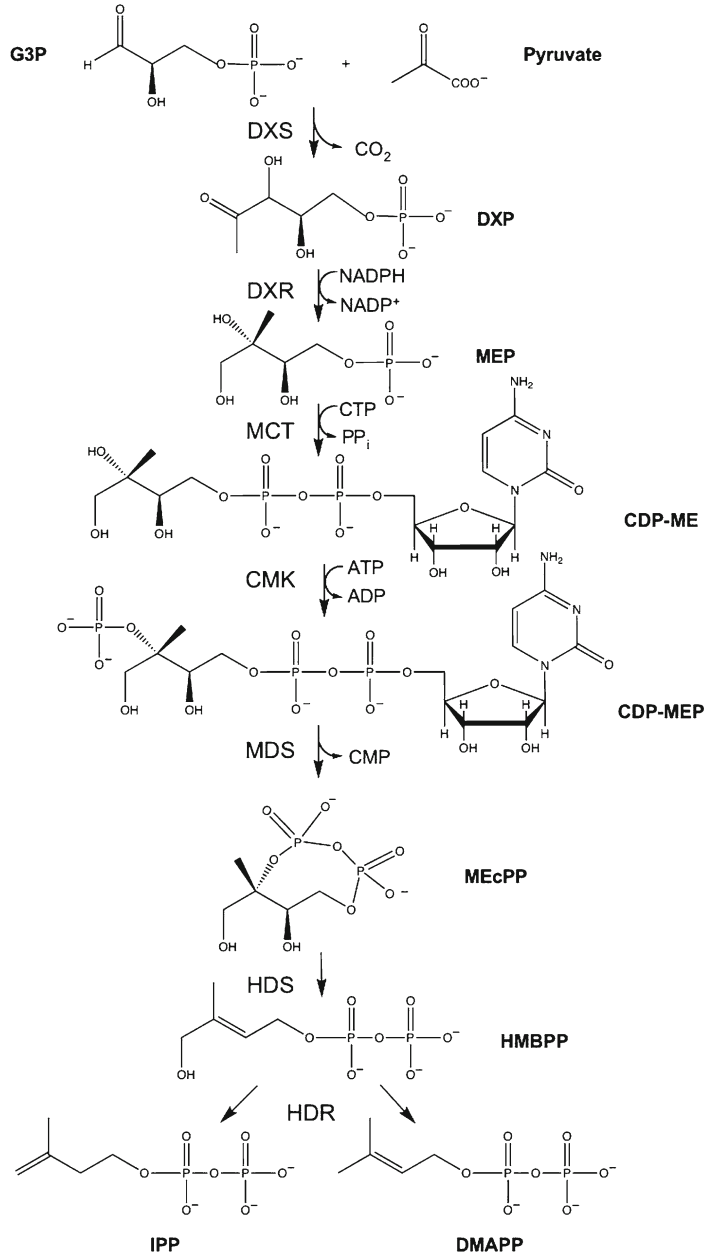

Fig. 1.4 Steps of the MEP pathway leading to the formation of IPP and DMAPP from pyruvate and D-glyceraldehyde 3-phosphate (*G3P*). Acronyms of enzymes and intermediates are described in the text and correspond to those suggested by Phillips et al. (2008)

is widely distributed in nature and is present in most eubacteria (see below).

The first reaction of the MEP pathway (Fig. 1.4) is catalyzed by the enzyme 1-deoxy-D-xylulose 5-phosphate (DXP) synthase (DXS) and involves the condensation of (hydroxyethyl)thiamin derived from pyruvate with the C1 aldehyde group of D-glyceraldehyde 3-phosphate to produce DXP. In the second step, an intramolecular rearrangement and reduction of DXP by the enzyme DXP reductoisomerase (DXR) yields 2-*C*-methyl-D-erythritol 4-phosphate (MEP). As described below, a different oxidoreductase enzyme with a DXR-like (DRL) activity was

recently found in a reduced number of bacteria (Sangari et al. 2010). MEP produced by DXR or DRL is then converted to 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate by the sequential action of the enzymes 2-*C*-methyl-D-erythritol 4-phosphate cytidylyltransferase (MCT), 4-(cytidine 5′-diphospho)-2-*C*-methyl-D-erythritol kinase (CMK), and 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate synthase (MDS). An opening reduction of MEcPP ring is catalyzed by the enzyme 4-hydroxy-3-methylbut-2-enyl diphosphate (HMBPP) synthase (HDS), which forms HMBPP. Finally, the enzyme HMBPP reductase (HDR) catalyzes the simultaneous formation of IPP and DMAPP. The functional and structural properties of the different MEP pathway enzymes have recently been reviewed by Hunter (2007).

The MVA pathway (Fig. 1.5) starts with the sequential condensation of three molecules of acetyl-CoA to yield 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) catalyzed by the enzymes acetoacetyl-CoA thiolase (AACT) and HMG-CoA synthase (HMGS). HMG-CoA is then converted to MVA in a functionally irreversible reaction catalyzed by HMG-CoA reductase (HMGR). MVA is sequentially phosphorylated and decarboxylated to generate IPP by the enzymes mevalonate kinase (MVK), 5-phosphomevalonate kinase (PMDK), and 5-diphosphomevalonate decarboxylase (DPMD). As indicated below, some Archaea species contain an alternative version of the MVA pathway involving the last two steps leading to the formation of IPP.

In contrast to the MEP pathway that simultaneously produces both IPP and DMAPP, the MVA pathway can only produce IPP. Thus, the isomerization of IPP to DMAPP, catalyzed by IPP isomerase (IDI) (Fig. 1.1), is an essential reaction in those organisms containing the MVA pathway. Two types of IPP isomerase are currently known. The type I enzyme is found in many eubacteria (including *E. coli*), yeast, and mammals and has been extensively characterized at structural and functional level (Durbecq et al. 2001; Wouters et al. 2003). The type II enzyme was discovered recently in *Streptomyces* sp. (Kaneda et al. 2001) and is known to be present in Archaea and some bacteria, but not in plants and animals (Kuzuyama and Seto 2003; Laupitz et al. 2004). Type I and type II IDI show no sequence similarity and have different cofactor requirements (Kaneda et al. 2001). The crystal structure of type II IDI from *B. subtilis* and *Thermus thermophilus* has recently been resolved by Steinbacher et al. (2003a) and de Ruyck et al. (2008), respectively.

1.4 Distribution of the MEP and MVA Pathways in Microorganisms

The large number of currently available sequenced genomes is providing a clear picture about the distribution of the MEP and MVA pathways in prokaryotic organisms. The distribution of both pathways, as well as that of type I and type II IDIs, in microorganisms belonging to representative groups is shown in Table 1.2. The archaeal genomes sequenced to date have exclusively revealed the presence of genes encoding MVA pathway enzymes. However, with the exception of some *Sulfolobus* species, the genomic analyses have failed to identify the full set of MVA pathway genes in the rest of species. In particular, the genes encoding PMVK and DPMD (Fig. 1.5) are absent in most archaebacteria (Boucher et al. 2004; Lombard and Moreira 2011). In these organisms, the conversion of phosphomevalonate to IPP is achieved through the operation of an alternative route involving the formation of isopentenyl phosphate from phosphomevalonate by phosphomevalonate decarboxylase (PMVD) and further conversion to IPP by IP kinase (IPK) (Fig. 1.5) (Grochowski et al. 2006). Although IPK has been characterized at the biochemical (Chen and Poulter 2010) and structural level (Mabanglo et al. 2010), the PMVD activity is still speculative and needs biochemical confirmation.

As shown in Table 1.2, most eubacteria contain the MEP pathway. Species containing the MVA pathway include the spirochaete *Borrelia burgdorferi* and the Gram-positive cocci *Staphylococcus aureus* and *Streptococcus pneumoniae*. Among the few bacterial species