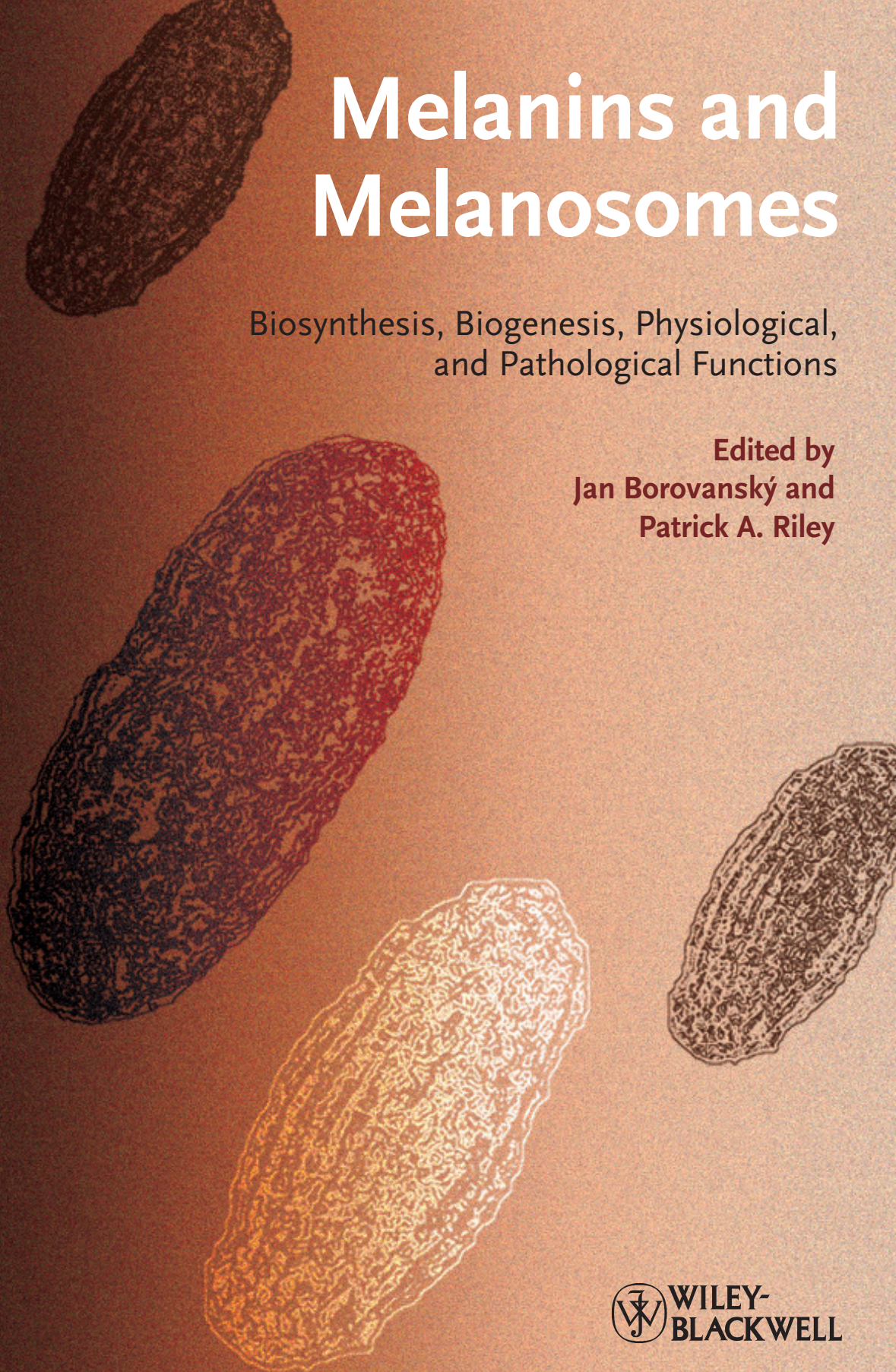


Melanins and Melanosomes

Biosynthesis, Biogenesis, Physiological,
and Pathological Functions

Edited by
Jan Borovanský and
Patrick A. Riley



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Melanins and Melanosomes

Biosynthesis, Biogenesis, Physiological,
and Pathological Functions

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Cover

The cover design is intended to reflect the two principal elements of the book: melanins and melanosomes. The melanins are represented by the graduated background pigment spanning the range of colours from essentially black eumelanin, through reddish to paler shades, representing pheomelanin. The cellular organelles responsible for the production, containment and distribution of melanins, the melanosomes, are represented by symbolic images based on their electron microscopic appearance.

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Dedication

“All nature is but art, unknown to thee;
All chance, direction which thou canst not see”

Alexander Pope: *An Essay on Man*

Were we to ascribe to chance the existence of this volume we should have to begin with the moment in July 1952 when Professor A.F. Richter, Head of the Second Institute of Medical Chemistry at Charles University in Prague, opened a dust-covered cabinet from which he took, apparently at random, a bottle containing a dark powder and handed it to a young assistant with the words: “Young man, study the contents of this flask.” The assistant was Jiri Duchon and the label on the flask read: “Human melanosaoma, prepared by H. Waelsch.”

Jiri Duchon was born on 27 July 1927, the only son of an eminent scientist. On graduating in medicine in 1952 he joined Richter’s laboratory and his careful analysis of the sample given to him set him upon the course of studies that were to occupy him for the rest of his life. He defended his PhD thesis in 1962 on the topic of “Urinary melanogens in melanoma” and he subsequently made many important contributions to quantitative analysis of the products of melanogenesis. In recognition of his early work, Jiri Duchon was awarded a Roosevelt Fellowship that enabled him to spend 15 months at Harvard in the laboratory of T.B. Fitzpatrick. This was in 1967–1968 when he met and established a friendship with Makoto Seiji who had just developed the methods for melanosome isolation. On his return to Prague, Jiri Duchon set about improving the isolation technique and analyzing these newly discovered organelles. Under his direction and inspiration the Prague laboratory became the leading European center for the detailed biochemical investigation of melanosomes. Jiri Duchon was Head of the Institute for 26 years and many of his collaborators have continued to contribute significantly to the field of study that he promoted.

Professor Duchon was an internationally recognized and highly respected member of the pigment cell fraternity, and was elected an Honorary Member of the European Society for Pigment Cell Research in 1998. It was partly in his honor that the scientific session on “Melanin and Melanosomes” was arranged at the Federation of European Biochemical Societies (FEBS) Congress in 2009, but

tragically he was taken ill on the very day of the Symposium. He was full of encouragement for the project that grew out of the meeting, namely that of publishing a definitive volume devoted to the subject of his academic endeavors, but died on 2 November 2009, long before it was completed.

In recognition of his seminal role in the events that led to the production of this book we dedicate this volume to Jiri Duchon with affectionate remembrance of a fine scientist, an inspirational teacher, a kindly and cultivated companion, and a true friend.



Professor Jiri Duchon MD, PhD, DrSc (1927–2009)
(Photograph by K. Meister)

Contents

| | | |
|----------|---|-----------|
| | Dedication | V |
| | Preface | XV |
| | List of Contributors | XIX |
| 1 | History of Melanosome Research | 1 |
| | <i>Jan Borovanský</i> | |
| 1.1 | Introduction | 1 |
| 1.2 | Melanosome Research in the Pre-Seiji Era | 1 |
| 1.3 | Melanosome Research in the Seiji Era | 5 |
| 1.3.1 | Terminology of Melanosomes | 5 |
| 1.3.2 | Ultrastructural and Histochemical Studies | 6 |
| 1.3.3 | Biochemical Studies | 7 |
| 1.4 | Melanosome Research in the Post-Seiji Era | 9 |
| 1.5 | Other Historical Aspects | 11 |
| | Acknowledgments | 12 |
| | References | 13 |
| 2 | Classical and Nonclassical Melanocytes in Vertebrates | 21 |
| | <i>Sophie Colombo, Irina Berlin, Véronique Delmas, and Lionel Larue</i> | |
| 2.1 | Definition of Melanogenic Cells | 21 |
| 2.2 | Distribution and Function of Melanogenic Cells | 24 |
| 2.2.1 | Classical Melanocytes | 25 |
| 2.2.1.1 | Melanocytes in the Epidermis | 25 |
| 2.2.1.2 | Melanocytes in the Dermis | 27 |
| 2.2.1.3 | Melanophores in Lower Vertebrates | 28 |
| 2.2.2 | Nonclassical Melanocytes | 28 |
| 2.2.2.1 | Melanocytes of the Eye | 28 |
| 2.2.2.2 | Melanocytes of the Inner Ear | 31 |
| 2.2.2.3 | Melanocytes of the Heart | 33 |
| 2.2.2.4 | Melanocytes of the Brain and Neuromelanins | 36 |
| 2.2.2.5 | Melanin in Adipose Tissue | 37 |
| 2.3 | Embryonic Development of Melanogenic Cells | 37 |

| | | |
|----------|--|-----------|
| 2.3.1 | Classical Melanocytes | 38 |
| 2.3.1.1 | Early Determined Melanoblasts: The Dorsolateral Pathway | 38 |
| 2.3.1.2 | Late Determined Melanoblasts: A Common Origin with SCPs and the Dorsoventral Migratory Pathway | 40 |
| 2.3.2 | Nonclassical Melanocytes | 41 |
| 2.3.2.1 | Melanocytes of the Murine Eye | 42 |
| 2.3.2.2 | Melanocytes of the Murine Heart | 44 |
| 2.3.2.3 | Other Nonclassical Murine Melanocytes | 45 |
| 2.3.2.4 | Other Organisms | 45 |
| 2.4 | Transfer of Melanin from Classical and Nonclassical Melanocytes | 45 |
| 2.4.1 | Melanosome Transport | 46 |
| 2.4.2 | Melanosome Transfer | 46 |
| 2.4.2.1 | Melanosome Transfer from Classical Melanocytes | 47 |
| 2.4.2.2 | Transfer of Melanin from Nonclassical Melanocytes | 51 |
| | References | 52 |
| 3 | Biological Chemistry of <i>o</i>-Quinones | 63 |
| | <i>Patrick A. Riley, Christopher A. Ramsden, and Edward J. Land</i> | |
| 3.1 | General Biological Significance of <i>o</i> -Quinones | 63 |
| 3.1.1 | Antibiosis | 63 |
| 3.1.2 | Defensive Secretions | 64 |
| 3.1.3 | Balanid Adhesion | 64 |
| 3.1.4 | Cuticular Hardening in Insects | 65 |
| 3.1.5 | Pigmentation | 65 |
| 3.2 | <i>o</i> -Quinone Reactivity | 66 |
| 3.2.1 | Structure and Reactivity | 66 |
| 3.2.2 | Reduction | 68 |
| 3.2.3 | Addition Reactions: Intermolecular addition | 71 |
| 3.2.4 | Polymerization | 71 |
| 3.2.5 | Intramolecular Addition (Cyclization) | 72 |
| 3.2.6 | Addition–Elimination (Substitution) Reactions | 73 |
| 3.3 | Role of <i>o</i> -Quinones in Melanogenesis | 74 |
| 3.3.1 | Nonenzymatic Formation of Melanogenic Intermediates | 74 |
| 3.3.1.1 | Contributions from Pulse Radiolysis to the Chemistry of Eumelanogenesis and Pheomelanogenesis | 74 |
| 3.3.2 | Balance between Eumelanogenesis and Pheomelanogenesis | 78 |
| 3.3.3 | Control of Melanogenesis: Phase I Melanogenesis | 78 |
| 3.3.4 | Tyrosinase Activation | 78 |
| 3.3.5 | Tyrosinase Inactivation | 79 |
| | References | 83 |
| 4 | Biosynthesis of Melanins | 87 |
| | <i>José Carlos García-Borrón and M. Concepción Olivares Sánchez</i> | |
| 4.1 | Introduction | 87 |
| 4.2 | Raper–Mason Pathway | 88 |

| | | |
|----------|---|------------|
| 4.2.1 | Phase I Melanogenesis: The Proximal Raper–Mason Pathway—From L-tyrosine to L-dopachrome | 88 |
| 4.2.2 | Distal Melanogenic Steps: From L-Dopachrome to Eumelanins | 90 |
| 4.2.3 | Biosynthesis of Pheomelanins | 91 |
| 4.3 | Structural and Functional Properties of the Melanogenic Enzymes | 92 |
| 4.3.1 | Structure of Tyrosinase and Related Proteins | 92 |
| 4.3.2 | Catalytic Cycle of Tyrosinase | 95 |
| 4.3.2.1 | Cresolase (Tyrosine Hydroxylase) Reaction Cycle | 96 |
| 4.3.2.2 | Catecholase (Dopa Oxidase) Reaction Cycle | 98 |
| 4.3.3 | Dct/Tyrp2 | 98 |
| 4.3.4 | Tyrp1 | 100 |
| 4.3.5 | Other Melanosomal Proteins | 101 |
| 4.4 | Regulation of the Melanogenic Pathway | 102 |
| 4.4.1 | Eumelanogenesis versus Pheomelanogenesis: Regulation of the Type of Melanin Pigments | 102 |
| 4.4.2 | Regulation of the Amount of Pigment | 104 |
| 4.4.2.1 | Regulation of Tyrosinase Levels | 104 |
| 4.4.2.2 | Control of Tyrosinase-Specific Activity | 106 |
| 4.5 | Conclusions and Perspectives | 107 |
| | Acknowledgments | 109 |
| | References | 109 |
| 5 | Inhibitors and Enhancers of Melanogenesis | 117 |
| | <i>Alain Taïeb, Muriel Cario-André, Stefania Briganti, and Mauro Picardo</i> | |
| 5.1 | Introduction | 117 |
| 5.1.1 | Melanin Biochemistry | 118 |
| 5.1.1.1 | Melanin Biosynthesis | 118 |
| 5.1.1.2 | Tyrosinase Maturation and Degradation | 118 |
| 5.1.1.3 | Catalytic Site | 119 |
| 5.1.2 | Paracrine Signaling and Regulation of Epidermal Melanogenesis | 119 |
| 5.1.3 | Methods of Study | 120 |
| 5.2 | Depigmenting Agents | 121 |
| 5.2.1 | Agents Acting Prior to Melanin Synthesis | 121 |
| 5.2.1.1 | Transcriptional Inhibition of Melanogenic Enzymes | 121 |
| 5.2.1.2 | Post-Translational Modification of Melanogenic Enzymes | 128 |
| 5.2.1.3 | Increased Tyrosinase Ubiquitination | 129 |
| 5.2.2 | Agents Acting During Melanin Synthesis | 129 |
| 5.2.2.1 | Interference with Tyrosinase | 129 |
| 5.2.2.2 | TRP-2 Modulation | 136 |
| 5.2.2.3 | Interference with Byproduct Production (Antioxidant and Reducing Agents) | 136 |
| 5.2.2.4 | Interference with the Melanogenic Pathway | 138 |
| 5.2.2.5 | Peroxidase Inhibitors | 139 |
| 5.2.3 | Agents Acting After Melanin Synthesis | 139 |

| | | |
|----------|--|------------|
| 5.2.3.1 | Inhibitors of Melanosome Transfer | 139 |
| 5.2.3.2 | Acceleration of Epidermal Turnover | 141 |
| 5.3 | Enhancers of Melanogenesis | 143 |
| 5.3.1 | Activation Through Receptor Mechanisms | 144 |
| 5.3.1.1 | Melanotropic Peptides | 144 |
| 5.3.1.2 | Cytokines and Growth Factors | 145 |
| 5.3.2 | Non-Receptor-Mediated Activation | 147 |
| 5.3.2.1 | Forskolin and cAMP | 147 |
| 5.3.2.2 | Oligonucleotides and p53 Activation | 147 |
| 5.3.2.3 | Piperin | 147 |
| 5.3.2.4 | Lipids (Sphingolipids and Prostaglandins) | 147 |
| 5.3.2.5 | Phospholipase A ₂ | 148 |
| 5.3.2.6 | PPAR Activators | 148 |
| 5.3.2.7 | Psoralens and Photosensitizing Agents | 148 |
| | References | 149 |
| 6 | Structure of Melanins | 167 |
| | <i>Shosuke Ito, Kazumasa Wakamatsu, Marco d'Ischia, Alessandra Napolitano, and Alessandro Pezzella</i> | |
| 6.1 | Introduction | 167 |
| 6.2 | Classification and General Properties of Melanins | 168 |
| 6.3 | Biosynthetic Studies | 169 |
| 6.3.1 | Early Stages of Melanogenesis | 169 |
| 6.3.2 | Late Stages of Eumelanogenesis | 171 |
| 6.3.3 | Late Stages of Pheomelanogenesis | 174 |
| 6.3.4 | Concept of Mixed Melanogenesis | 175 |
| 6.4 | Degradative Studies | 176 |
| 6.4.1 | Eumelanins | 176 |
| 6.4.2 | Pheomelanins | 178 |
| 6.5 | Analysis of Eumelanins and Pheomelanins | 180 |
| 6.6 | Conclusions | 180 |
| | References | 181 |
| 7 | Properties and Functions of Ocular Melanins and Melanosomes | 187 |
| | <i>Małgorzata Różanowska</i> | |
| 7.1 | Introduction | 187 |
| 7.2 | Biogenesis of Ocular Melanosomes and Melanogenesis | 187 |
| 7.3 | Melanin Content in Pigmented Structures of the Eye | 190 |
| 7.3.1 | Melanin Content in the RPE | 190 |
| 7.3.2 | Melanin Content in the Choroid | 193 |
| 7.3.3 | Melanin Content in the Iris | 193 |
| 7.4 | Structure of Ocular Melanosomes | 194 |
| 7.4.1 | Morphology of Ocular Melanosomes | 195 |
| 7.4.2 | Molecular Composition of Ocular Melanosomes | 196 |
| 7.4.2.1 | Melanosomal Proteins | 196 |

| | | |
|----------|--|------------|
| 7.4.2.2 | Melanosomal Lipids | 197 |
| 7.5 | Role of Ocular Melanin as a Broadband Optical Filter | 198 |
| 7.5.1 | Role of the Iris as a Filter of Light | 198 |
| 7.5.2 | Role of Melanin in Light Transmission Through the RPE and Choroid | 200 |
| 7.6 | Antioxidant Properties of Ocular Melanin | 201 |
| 7.6.1 | Scavenging of Free Radicals | 201 |
| 7.6.2 | Quenching of Electronically Excited States of Photosensitizers and Singlet Oxygen | 203 |
| 7.6.3 | Sequestration of Redox-Active Metal Ions | 206 |
| 7.6.4 | Testing Protective Effects of Ocular Melanin in Cultured Cells | 207 |
| 7.7 | Pro-Oxidant Properties of Ocular Melanosomes | 209 |
| 7.7.1 | Generation of ROS and Oxidation of Cellular Reductants | 210 |
| 7.7.2 | Pro-Oxidant Effects of Interactions of Melanosomes with Metal Ions | 213 |
| 7.7.3 | Cytotoxic Properties of Aged RPE Melanin Granules and Their Potential Consequences for Retinal Aging and AMD | 214 |
| 7.8 | Other Properties of Ocular Melanosomes and Their Implications | 216 |
| 7.9 | Conclusions | 217 |
| | References | 218 |
| 8 | Biological Role of Neuromelanin in the Human Brain and Its Importance in Parkinson's Disease | 225 |
| | <i>Kay L. Double, Wakako Maruyama, Makoko Naoi, Manfred Gerlach, and Peter Riederer</i> | |
| 8.1 | What are Neuromelanins? | 225 |
| 8.2 | Phylogenetic Development of Neuromelanin | 227 |
| 8.3 | Development and Metabolism of Neuromelanin | 227 |
| 8.4 | Structure of Neuromelanin | 231 |
| 8.5 | Biological Role of Neuromelanin in the Human Brain | 232 |
| 8.6 | Is Neuromelanin Involved in Neurological Disease? | 233 |
| 8.7 | Effects of Neuromelanin <i>In Vitro</i> and <i>In Vivo</i> | 235 |
| 8.7.1 | Mechanisms of Neuromelanin Cytotoxicity | 235 |
| 8.7.2 | Neuromelanin Effects on Mitochondrial Function | 237 |
| 8.7.3 | Neuromelanin Effects on the UPS | 239 |
| 8.7.4 | Comparison of the Cytotoxicity of Neuromelanin with Synthetic DA-M | 239 |
| 8.8 | Conclusions | 241 |
| | Acknowledgments | 241 |
| | References | 241 |
| 9 | Biogenesis of Melanosomes | 247 |
| | <i>Cédric Delevoeye, Francesca Giordano, Michael S. Marks, and Graça Raposo</i> | |
| 9.1 | Introduction | 247 |
| 9.2 | Melanosomes: Intracellular Organelles Specialized in Melanin Synthesis | 249 |

| | | |
|-----------|---|------------|
| 9.2.1 | Melanosomes Are Unique Organelles That Develop through Different Stages | 249 |
| 9.2.2 | Melanosomal Components | 251 |
| 9.3 | Endocytic System and Formation of Melanosomes | 254 |
| 9.3.1 | Organelles of the Endocytic Pathway | 254 |
| 9.3.2 | Melanosomes Are LROs but Are Distinct from Lysosomes | 256 |
| 9.3.3 | Pmel17 and Generation of Early-Stage Melanosomes | 259 |
| 9.3.3.1 | Pmel17 Structure | 259 |
| 9.3.3.2 | Pmel17 Forms the Fibrillar Matrix upon Which Melanins Deposit | 259 |
| 9.3.3.3 | Pmel17 Biosynthesis and Amyloid Formation | 260 |
| 9.3.3.4 | Functional Importance of Fibrillar Melanosomes | 262 |
| 9.3.4 | OA Type 1 and Melanosome Biogenesis | 263 |
| 9.3.5 | Origin of the Melanosome | 263 |
| 9.3.5.1 | Early-Stage Melanosomes Originate within the Endocytic Pathway | 263 |
| 9.3.5.2 | Melanosomes Do Not Originate from the ER | 265 |
| 9.3.5.3 | Melanosomes Segregate from the Endocytic Pathway beyond Stage I Melanosomes | 266 |
| 9.3.5.4 | Components of Mature Melanosomes Are Sorted from Distinct Endosomal Intermediates | 267 |
| 9.4 | Melanosome Maturation: Cargo Sorting to Mature Melanosomes | 269 |
| 9.4.1 | Griscelli Syndrome and CHS | 269 |
| 9.4.2 | HPS | 271 |
| 9.4.2.1 | Adaptor Protein (AP) Complexes | 271 |
| 9.4.2.2 | BLOC Complexes | 273 |
| 9.4.3 | Molecular Motors and the Cytoskeleton | 276 |
| 9.4.4 | SNAREs, Rabs, and Other Regulators | 277 |
| 9.4.5 | Lipids | 279 |
| 9.5 | Conclusions | 279 |
| | Acknowledgments | 280 |
| | References | 281 |
| 10 | Transport and Distribution of Melanosomes | 295 |
| | <i>Mireille Van Gele and Jo Lambert</i> | |
| 10.1 | Introduction | 295 |
| 10.2 | Model Systems to Study Pigment Transport | 296 |
| 10.2.1 | Melanophores from Fish and Amphibians | 296 |
| 10.2.2 | Mammalian Melanocytes | 298 |
| 10.2.3 | RPE Cells | 299 |
| 10.3 | Intracellular Melanosome Transport | 299 |
| 10.3.1 | Microtubule-Based transport | 300 |
| 10.3.1.1 | Kinesin and Dynein | 300 |
| 10.3.2 | Actin-Based Transport | 301 |
| 10.3.2.1 | MYO5A | 301 |
| 10.3.2.2 | RAB27A | 302 |

| | | |
|-----------|---|------------|
| 10.3.2.3 | MLPH | 303 |
| 10.3.2.4 | RAB27A–MLPH–MYO5A Tripartite Protein Complex | 304 |
| 10.3.2.5 | RAB27A as a New MITF Target Gene | 306 |
| 10.4 | Melanosome Motility in RPE: The Rab27a–Myrip–Myo7a Tripartite Complex | 307 |
| 10.5 | Melanosome Transfer | 309 |
| 10.5.1 | Modes of Transfer | 309 |
| 10.5.1.1 | Cytophagocytosis | 309 |
| 10.5.1.2 | Exocytosis | 310 |
| 10.5.1.3 | Filopodial-Phagocytosis Model | 311 |
| 10.5.2 | Molecular Players | 312 |
| 10.5.2.1 | PAR-2 and KGF | 312 |
| 10.5.2.2 | Adhesion Molecules: Cadherins and Lectins | 313 |
| 10.6 | Fate of Melanin in the Keratinocyte | 313 |
| 10.7 | Conclusions | 315 |
| | Acknowledgments | 316 |
| | References | 316 |
| 11 | Genetics of Melanosome Structure and Function | 323 |
| | <i>Vincent J. Hearing</i> | |
| 11.1 | Introduction | 323 |
| 11.2 | Genes Involved in Melanoblast Development, Migration, and Specification | 324 |
| 11.3 | Genes Involved in Melanocyte Differentiation, Survival, and Proliferation | 325 |
| 11.4 | Genes Involved in Regulating Melanocyte Function | 327 |
| 11.4.1 | Regulation of Constitutive Skin, Hair, and Eye Color | 330 |
| 11.4.2 | Hypopigmentation | 332 |
| 11.4.3 | Hyperpigmentation | 332 |
| 11.5 | Genes Involved in the Biogenesis of Melanosomes and Other Lysosome-Related Organelles | 333 |
| 11.6 | Genes Involved in Melanin Production | 334 |
| 11.7 | Genes Involved in Melanosome Movement, Transfer, and Distribution | 336 |
| 11.7.1 | Movement | 336 |
| 11.7.2 | Transfer | 337 |
| 11.7.3 | Distribution | 337 |
| 11.8 | Conclusions | 338 |
| | References | 338 |
| 12 | Physiological and Pathological Functions of Melanosomes | 343 |
| | <i>Jan Borovanský and Patrick A. Riley</i> | |
| 12.1 | Tissue Concentration of Melanosomes | 343 |
| 12.2 | Melanosome Properties and Functions Are Determined by Their Chemical Composition | 344 |

| | | |
|-----------|--|------------|
| 12.3 | Functional Microanatomy of the Melanosome | 346 |
| 12.4 | Melanosomes as Centers of Free Radical Activity | 350 |
| 12.4.1 | Free Radical Nature of Melanins | 350 |
| 12.4.2 | Radicals and Reactive Species Associated with Melanogenesis | 352 |
| 12.4.3 | Possible Role of Protein-Bound Dopa | 355 |
| 12.4.4 | Melanosomes as a Therapeutic Target | 356 |
| 12.5 | Melanosomes as Energy Transducers | 358 |
| 12.5.1 | Photon/Phonon Conversion | 359 |
| 12.5.2 | Photochemical Reactions | 360 |
| 12.5.3 | Sound/Heat Conversion | 360 |
| 12.6 | Melanosomes and Metal Ions | 360 |
| 12.7 | Affinity of Melanosomes for Polycyclic and Other Compounds | 364 |
| 12.7.1 | Melanoma Detection and Treatment | 365 |
| 12.7.2 | Participation of Melanosomes in Chemoresistance | 366 |
| 12.7.3 | Long-Term Deposition of Compounds in Melanosomes | 367 |
| 12.8 | Exploitation of Melanosomal Proteins and Melanin as Specific Targets in Melanoma Therapy | 368 |
| 12.9 | Conclusions | 370 |
| | Acknowledgments | 370 |
| | References | 371 |
| 13 | Dysplastic Nevi as Precursor Melanoma Lesions | 383 |
| | <i>Stanislav Pavel, Nico P.M. Smit, and Karel Pizinger</i> | |
| 13.1 | Nevi as Risk Factors for Melanoma | 383 |
| 13.1.1 | Development of Melanocytic Nevi | 383 |
| 13.1.2 | Description of Dysplastic Nevi | 384 |
| 13.2 | Dysplastic Nevi as Precursor Lesions of Melanoma | 384 |
| 13.3 | Cytological Differences between Normal Skin Melanocytes and Dysplastic Nevus Cells: Melanosomal and Mitochondrial Aberrations | 386 |
| 13.4 | Metabolic Differences between Normal Skin Melanocytes and Dysplastic Nevus Cells: Preference for Pheomelanogenesis in Dysplastic Nevus Cells | 387 |
| 13.5 | Pheomelanogenesis as a Possible Cause of Intracellular Oxidative Imbalance | 388 |
| 13.6 | Dysplastic Nevus Cells as Senescent Cells | 389 |
| 13.7 | Are Dysplastic Nevus Cells a Class of Cells Exhibiting a Mutator Phenotype? | 389 |
| | References | 391 |
| | Index | 395 |

Preface

“To that small part of ignorance that we arrange and classify we give the name knowledge”

Ambrose Bierce

This book is entitled *Melanin and Melanosomes*, and is about pigment and pigmentation. It is important, however, that we bear in mind that, while the primary function of melanocytes is the production of pigment in melanosomes, these cells have other attributes and perform other significant functions. Some of these are well recognized, such as the involvement of the retinal pigment epithelium in photoreceptor physiology (detailed in Chapter 7). Another interesting possibility is that melanogenesis may be the source of some of the substrate for dopamine synthesis [1], and melanocytes may have other important neuroendocrine functions as pro-propiomelanocortin processing cells and a source of prostaglandin D synthase (reviewed by Takeda *et al.* [2]). Some of these actions may go some way to explaining the remarkable anatomical distribution of melanocytes, often in locations that are not illuminated, such as the leptomeninges.

However, this volume is devoted to melanin and melanosomes, and is primarily concerned with vertebrate, especially human, pigmentation. We include melanin that is formed by oxidative processes that are enzymatically catalyzed in specialized cells, both the neural crest-derived dendritic melanocytes (named “classical” melanocytes in Chapter 2) and optic cup-derived retinal pigment epithelial cells (“nonclassical” melanocytes), as well as melanin generated by other oxidative pathways, such as the neuromelanin of the midbrain. The importance of this latter pigment, particularly in relation to Parkinson’s disease, is set out in Chapter 8.

The enzymatically generated melanin in vertebrates is synthesized and deposited in specialized intracellular organelles, the melanosomes, and this book concentrates on the many aspects of the formation and functions of these organelles.

The melanosome is a highly specialized organelle, the history of which owes much to the early work at Charles University under the aegis of Jiri Duchon (1927–2009), to whom this book is dedicated.

Many of the important properties of melanosomes were established in Prague in the early 1970s by a series of investigations on isolated and purified preparations of this organelle, and investigators at Charles University have continued to

contribute significantly to the advancement of this field, and to follow the developments that have taken place in elucidating the structure of melanosomes and the complex biological roles in which they are implicated.

This volume grew from a combination of auspicious factors. In 2009, the 34th Congress of the Federation of European Biochemical Societies (FEBS) was organized in Prague. Naturally, with one of us (J.B.) on the Organizing Committee, one of the scientific sessions was devoted to melanosomes and, in the wake of the discussions at this meeting, it was felt that there was a significant body of new data relating to melanosomes that could usefully be assembled in a volume devoted exclusively to this organelle.

It was hoped that such an overview, by integrating the diverse aspects of current knowledge, might help to generate a new understanding of the biological role of melanosomes and stimulate novel research effort in this interesting area of study.

We have been fortunate in our publisher, Wiley-VCH, who recognized the timely nature of the proposed volume, and we thank our commissioning editor, Gregor Cicchetti, and his team, especially Anne Chassin du Guerny, for their help and encouragement in bringing the project to fruition.

Of course, our main thanks go to our panel of distinguished international contributors who have generously given of their time and expertise in preparing the chapters that we hope form a coherent picture of the up-to-date knowledge in the field.

Last, but not least, this book celebrates a long and fruitful collaboration between the Editors involving many visits between Charles University and University College London. It is a pleasure to acknowledge the assistance of the British Council in enabling these exchanges.

We had hoped initially to have the opportunity to arrange the order of the chapters in the light of their ultimate content so that overlapping areas were most rationally ordered to enable the volume to be read more or less in sequence while allowing the, perforce abundant, cross-references to act as a secondary web in a cohesive network. However, pressure of time prevented us from completing this task and readers may find it more convenient to skip between the various contributions according to their interests and predilections. In principle, although the topics are inextricably intertwined, we have elected to place the contributions devoted to melanin – its biosynthesis, chemistry, and properties – at the front of the book, and those dealing with melanosomes – their structure, biogenesis, distribution, and properties – in the following chapters.

The topic is put into chronological context by a historical Introduction in Chapter 1, in which Jan Borovanský traces the steps in the discovery of the melanosome, illustrated by portraits of the important investigators that took part in these exciting early studies.

As this book is directed largely at aspects of human pigmentation, Chapter 2 consists of a detailed overview by Sophie Colombo, Irina Berlin, Véronique Delmas, and Lionel Larue of the specialized cells in vertebrates in which melanin production in melanosomes takes place. In their contribution a distinction is made between “classical” and “nonclassical” melanocytes. Chapter 3, by Patrick Riley,

Christopher Ramsden, and Edward Land, emphasizes the central role of the generation and reactivity of *o*-quinones in melanogenesis, and is followed by Chapter 4 in which the biosynthesis of melanins is reviewed by José Carlos Garcia-Borrón and Conchita Olivares Sánchez. Chapter 5, by Alain Taieb, Muriel Cario-André, Stefania Briganti, and Mauro Picardo, comprises an analysis of inhibitors and enhancers of melanogenesis. The current understanding of the structure of melanins is then reviewed in Chapter 6 by Shosuke Ito, Kasumasa Wakamatsu, Marco d'Ischia, Alessandra Napolitano, and Alessandro Pezzella, and this is followed in Chapter 7 by a description of the properties and functions of ocular melanins and melanosomes by Małgorzata Rózanowska. Chapter 8, by Kay Double, Wakako Maruyama, Makako Naoi, Manfred Gerlach, and Peter Riederer, is devoted to the biological role of neuromelanin in the human brain and its importance in Parkinson's disease. Chapter 9 consists of a detailed review of the biogenesis of melanosomes by Cédric Delevoye, Francesca Giordano, Michael Marks, and Graça Raposo. This is followed in Chapter 10, by Mireille Van Gele and Jo Lambert, by a description of the transport and distribution of melanosomes. The genetics of melanosome structure and function are skillfully summarized in Chapter 11 by Vincent Hearing. Chapter 12, by Jan Borovanský and Patrick Riley, is devoted to the properties and functions of melanosomes, and, in Chapter 13, the abnormalities of melanosomes and melanogenesis in melanoma precursor lesions are discussed by Stan Pavel, Nico Smit, and Karel Pizinger.

We firmly believe that this compilation of expertise embodies a significant work of scholarship, and we sincerely hope that the combined wisdom embraced by this volume conveys both the breadth of detailed and exciting knowledge that currently exists about melanin and melanosomes, and also reveals those shadowed areas of doubt and ignorance that await illumination in the future.

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Patrick A. Riley
Jan Borovanský

References

- 1 Eisenhofer, G., Tian, H., Holmes, C., Matsunaga, J., Roffler-Tarlov, S., and Hearing, V.J. (2003) Tyrosinase: a developmentally specific major determinant of peripheral dopamine. *FASEB J.*, **17**, 1248–1255.
- 2 Takeda, K., Takahashi, N.-H., and Shibahara, S. (2007) Neuroendocrine functions of melanocytes: beyond the skin-deep melanin maker. *Tohoku J. Exp. Med.*, **211**, 201–221.

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1

History of Melanosome Research

Jan Borovanský

1.1

Introduction

Melanosomes were first proposed as specific organelles, unique to pigment cells, in a preliminary publication that appeared on 30 July 1960 [1]. An announcement had been made at the 21st Annual Meeting of the Society for Investigative Dermatology, at Miami Beach, Florida, USA on 13 June 1960 [2] and the news, that the chemical composition and enzyme activities in melanosomes and mitochondria are completely different, was considered to be of such significance that it appeared in a newspaper report (Figure 1.1). Similar data, with an emphasis on terminology, were published in 1963 [3].

This advance was the result of collaborative work between M. Seiji (1926–1982), at that time working at the Department of Dermatology, Harvard Medical School in Boston under the leadership of T.B. Fitzpatrick (1919–2003) (Figure 1.2), and H. Blaschko and M.S.C. Birbeck, with whom Dr Fitzpatrick established scientific cooperation during his tenure of a Commonwealth Fellowship at the Department of Biochemistry, Radcliffe Infirmary in Oxford.

The history of melanosome research can be formally divided into three parts: (i) the pre-Seiji era (prior to 1960), (ii) the Seiji era (1960–1982), and (iii) the post-Seiji era (1983–).

1.2

Melanosome Research in the Pre-Seiji Era

The first description of mammalian pigment cells was published by Gustav Simon in 1841 [4] who observed round and stellate pigment cells in the hair bulbs of pig embryos. It was preceded in 1838 by Purkyně's description of pigment in the cells of the substantia nigra, which not only drew attention to pigment granules, but also noted the rise in their numbers with age [5]. We have to admire these early reports because their authors, armed only with primitive light microscopes, were able to ascertain that melanin was not diffusely distributed in the cytoplasm

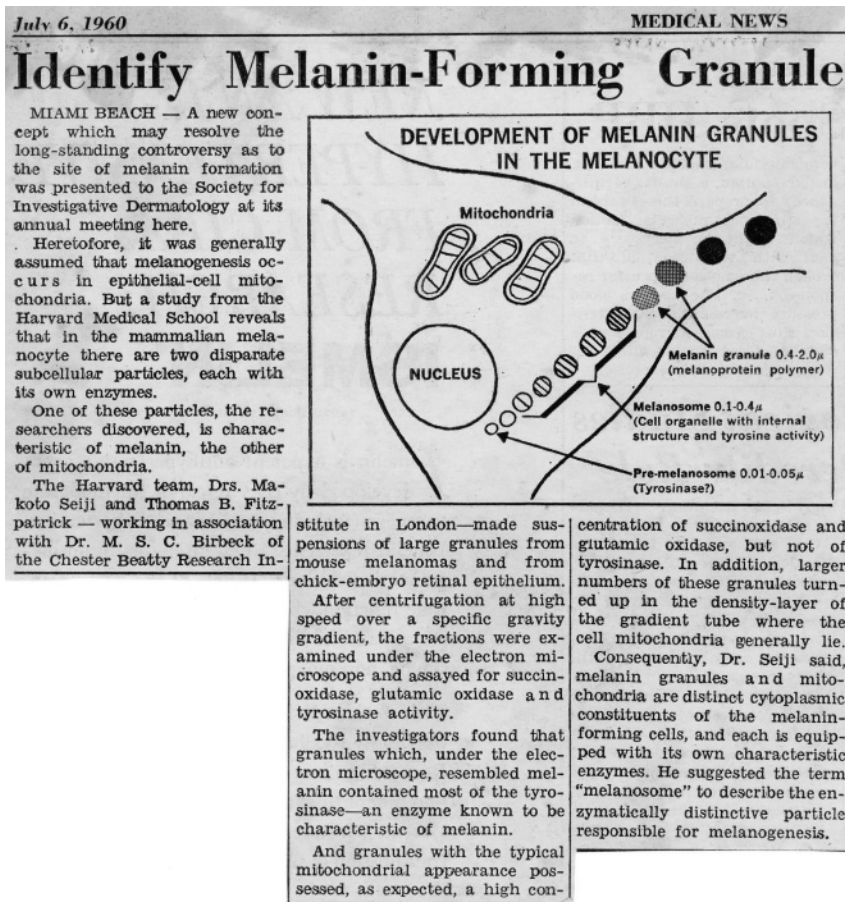


Figure 1.1 Announcement of the independent status of melanosome in *Medical News* on 5 July 1960.

of pigmented cells, but was present in the form of discrete aggregates [5, 6] (Figures 1.3 and 1.4).

Deciphering the old literature is problematical as authors often fail to distinguish between melanin (the pigment itself), melanoprotein (the natural melanin-protein complex), and melanin granules (the subcellular organelle). If the method of separation is not adequately described, it is difficult to be certain what material was studied and any conclusions can be misleading [8]. The lack of electron microscopic identification of isolated material led to many misinterpretations; for example, the “melanopseudoglobulin” studied by Greenstein *et al.* [9] was later shown to be melanosomes [10] and Bolt’s “melanoprotein” [11], widely used in biophysical studies, turned out to consist of damaged melanosomes [12]. Mason *et al.* [10] posed the question of whether melanin granules were particles with a specific structure or consisted of random aggregates of precipitated metabolic



Figure 1.2 Professor Makoto Seiji (left) and Professor Thomas B. Fitzpatrick (right) in 1972.

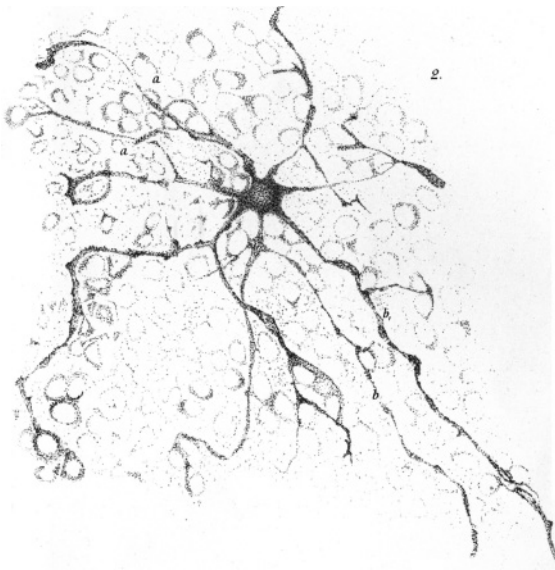


Figure 1.3 “Chromatophore” from donkey conjunctiva [7].

products. The introduction of electron microscopy was able to resolve this matter and Laxer *et al.* [13] were able to discern an inner ultrastructure in isolated melanosomes. The first clear pictures were obtained only in 1956 [14].

An avalanche of papers in subsequent years brought with it enormous amounts of information on the ultrastructure of melanosomes and its changes during

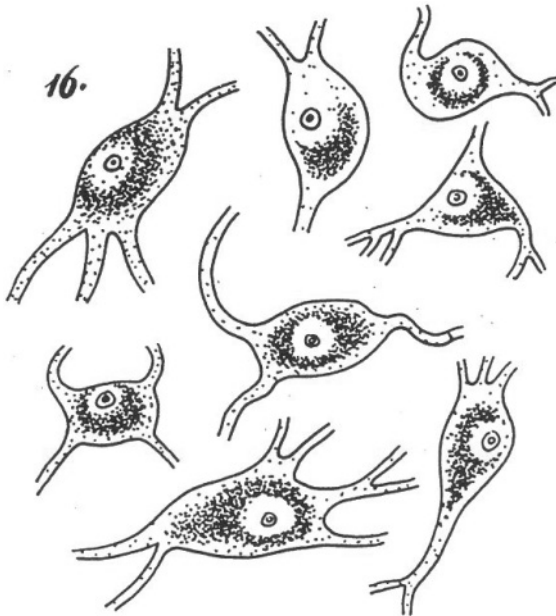


Figure 1.4 Cells of substantia nigra containing neuromelanin [5].

melanosome development (good examples are [15–17]). Other papers (reviewed in [18]) brought together ultrastructural and biochemical data that, in combination, laid the basis for the nomenclature of melanosomal ontogenesis.

By comparison with the morphological data, biochemical investigations of melanosomes were more modest, mainly due to the fact that ultrastructural data were derived from studies of intact cells or tissues, whereas biochemical research used samples prepared by relatively harsh preparative procedures. These samples sometimes consisted of melanins, or altered melanosomes, or their fragments, usually without any check of their nature or homogeneity [18].

The aim of researchers in the nineteenth century was not to prepare subcellular particles or native melanoproteins, but to separate the colored pigment (“Farbstoff” = melanin in the terminology of that time). The presence of protein in the isolated material was considered an unwanted contaminant [19]. Probably the first mild separation protocol was used by J.J. Berzelius [20]. He investigated pigment (melanosomes?) obtained from eye membranes by water extraction, and noticed its insolubility in acids and limited solubility in alkali. Similar mild extraction procedures were used by Landolt [21] and Mörner [22]. The early isolation procedures were reviewed by Waelsch [23]. He studied “natural melanin” from human melanoma metastases and horse choroids, confirmed the presence of protein attached to pigment, and suggested that melanin could be synthesized from the cyclic amino acids present in the protein moiety; this idea has not been

abandoned till now. Herrmann and Boss [24] demonstrated dopa oxidase activity in the fraction of melanin granules from ciliary bodies of cattle eyes, but, as their samples were contaminated with mitochondria, they demonstrated the presence of mitochondrial enzyme markers as well. In 1949, du Buy *et al.* concluded that melanosomes are modified mitochondria typical of pigment cells [25]. It is interesting that du Buy [26] and other authors [27] did not abandon the mitochondrial theory of melanosome origin even in 1963 (i.e., 2 years after the formulation of Seiji's melanosomal concept) and even published their papers in the same volume in which Seiji *et al.* published detailed confirmation of their model [28].

It is interesting that history has disregarded the contribution of Stein [29] who, several years before the work of Seiji *et al.*, using a separation procedure of his own, isolated melanin granules from ox choroids and analyzed their content not only of melanin, but also lipids, carbohydrates, RNA, and metals (including the pioneer finding of a high level of zinc), and concluded that the chemical composition of melanin granules is completely different from mitochondria.

The ability of melanin in melanin granules, isolated from Harding-Passey melanoma and from the ink sac of *Loligo opalescens*, to act as a cation exchanger [30], and the demonstration of free radical activity in melanin-containing tissues [31] also rank among the observations of the pre-Seiji era.

1.3

Melanosome Research in the Seiji Era

1.3.1

Terminology of Melanosomes

The demonstration of melanosomes as unique pigment cell organelles possessing developmental stages prompted the introduction of a system of terminology that reflected the characteristics of the various states. Until 1961 the common term for all varieties of these organelles was melanin (or pigment) granule [1, 2]. The first system of nomenclature [2] described three stages in the ontogenesis of melanosomes:

- i) Premelanosomes: spherical organelles.
- ii) Melanosomes: organelles with an internal structure and tyrosinase activity.
- iii) Melanin granules: melanoprotein polymer.

A second terminological system was proposed [3, 26] consisting of three developmental stages plus a final product. Thus:

- Stage I (first stage): biosynthesis of protein.
- Stage II (intermediate stage): biosynthesis of organelle.
- Stage III (late phase): biosynthesis of melanin.
- Final product: melanin granule.

These nomenclature systems introduced a certain degree of confusion, particularly as the term melanin granule had been used to describe pigment granules at any developmental stage. In an attempt to establish a consensus, Fitzpatrick *et al.* [32, 33] circulated a postal questionnaire seeking opinions about the adequacy of the terms in common use in pigment cell research and, with the approval of the participants of the Sixth International Pigment Cell Conference in 1965 in Sofia, Bulgaria, recommended the use of two terms:

- **Melanosome:** a discrete melanin-containing organelle in which melanization is complete as indicated by its almost uniform density by electron microscopy and the absence of demonstrable tyrosinase activity.
- **Premelanosome:** a term applied to all the stages in melanosome biogenesis that precede the fully developed state. Within the restrictions of this general definition, the premelanosomal stage might, at the discretion of the investigator, be subdivided into early, intermediate, and late phases.

The nomenclature in general use today does not adhere to any of the three systems outlined above, but is essentially a system proposed by Toda *et al.* [34–36] reflecting the earlier descriptions of Birbeck [37, 38] which employs the uniform term “melanosome” with a numerical indication (I–IV) of the degree its ontogenetic development.

However, in practice, chaos prevails. While the system of Toda *et al.* is widely–if somewhat erratically–used, some European authors refer, often incorrectly, to the stages proposed in the second system of nomenclature [3, 26] and some American authors tend to cite nomenclature introduced in their previous papers or those of their friends.

1.3.2

Ultrastructural and Histochemical Studies

The concept of subcellular biosynthesis and localization of melanins and melano-proteins in melanosomes was further confirmed by (i) autoradiographic evidence with [^3H]dopa and [$2\text{-}^{14}\text{C}$]dopa [39–43], (ii) incorporation of [$2\text{-}^{14}\text{C}$]dopa and monitoring radioactivity in subcellular fractions [44, 45], and (c) isolation of melanosomes and analysis of their chemical composition [46, 47].

Electron microscopy enabled the definition of the basic morphometric data of isolated melanosomes (i.e., their size, shape, and ultrastructural appearance). The most extensive data were published by Hach *et al.* [48, 49]. For discussion concerning the ultrastructural appearances of melanosomes, see Section 12.3 in Chapter 12.

Various pathological states may be manifested by changes in melanosome morphology. Mishima *et al.* [50] considered that melanosome polymorphism, such as changes in size, shape, ultrastructural matrix, the manner of melanin deposition, and the degree of melanosome maturation, as a criterion of molecular pathology that could find practical use in the differential diagnosis of various pigmentary disorders.