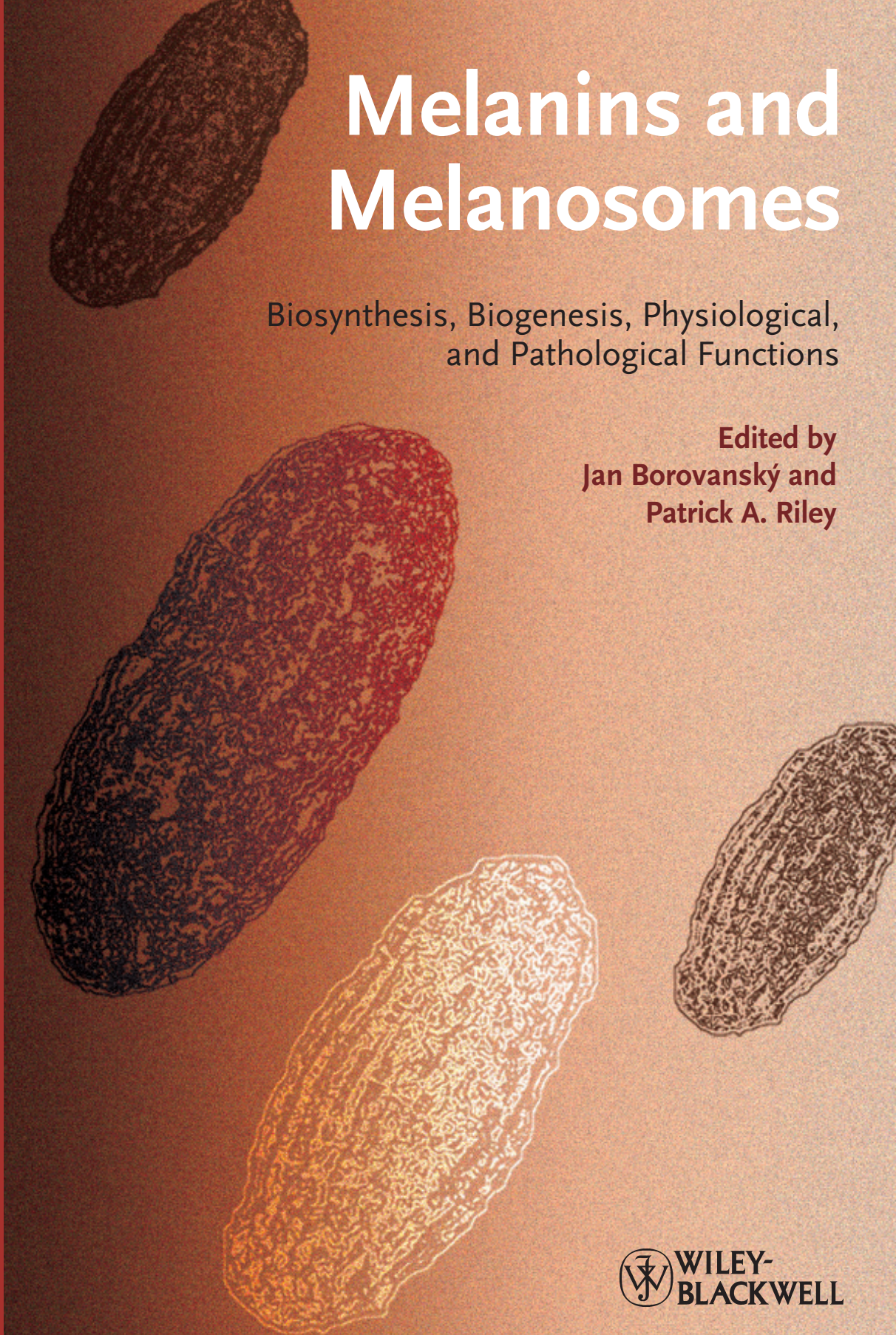
# Melanins and Melanosomes

## 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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**The Editors**

***Prof. Patrick A. Riley***
Em. Prof. of Cell Pathology
University College London
Grange Avenue
London N20 8AB
United Kingdom

***Prof. Jan Borovanský***
Charles University
1st Faculty of Medicine
Institute of Biochemistry and
Experimental Oncology
U Nemocnice 5
128 53 Praha 2
Czech Republic

**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 celluar 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 melanosarcoma, 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

# 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-propriomelanocortin 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 Taïeb, 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 Różanowska. 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.

March 2011

*Patrick A. Riley*
*Jan Borovanský*

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## List of Contributors

***Irina Berlin***
Institut Curie
Developmental Genetics of Melanocytes, INSERM U1021–CNRS UMR3347
Centre Universitaire
91405 Orsay
France

***Jan Borovanský***
Charles University
First Faculty of Medicine, Institute of Biochemistry and Experimental Oncology
U nemocnice 5
128 53 Prague 2
Czech Republic

***Stefania Briganti***
San Gallicano Dermatological Institute
Laboratory of Cutaneous Physiopathology
Elio Chianesi 53
00144 Rome
Italy

***Muriel Cario-André***
Université de Bordeaux
INSERM U1035
146 rue Léo Saignat
33076 Bordeaux
France

***Sophie Colombo***
Institut Curie
Developmental Genetics of Melanocytes, INSERM U1021–CNRS UMR3347
Centre Universitaire
91405 Orsay
France

***Marco d'Ischia***
University of Naples "Federico II"
Department of Organic Chemistry and Biochemistry
Via Cinthia 4
80126 Naples
Italy

***Cédric Delevoye***
Institut Curie
Centre de Recherche
Structure and Membrane Compartments
26 Rue d'Ulm
75248 Paris cedex 05
France

***Véronique Delmas***
Institut Curie
Developmental Genetics of Melanocytes, INSERM U1021–CNRS UMR3347
Centre Universitaire
91405 Orsay
France

***Kay L. Double***
University of New South Wales
Neuroscience Research Australia
Barker Street
Sydney, NSW 2031
Australia

***José Carlos García-Borrón***
University of Murcia
Department of Biochemistry and Molecular Biology
Campus de Espinardo
30100 Murcia
Spain

***Manfred Gerlach***
University of Würzburg
Clinical Neurobiology, Department of Child and Adolescence Psychiatry, Psychosomatics and Psychotherapy
Füchsleinstrasse 15
97080 Würzburg
Germany

***Francesca Giordano***
Institut Curie
Centre de Recherche
Structure and Membrane Compartments
26 Rue d'Ulm
75248 Paris cedex 05
France

***Vincent J. Hearing***
National Institutes of Health
Laboratory of Cell Biology
37 Convent Drive
Bethesda, MD 20892
USA

***Shosuke Ito***
Fujita Health University School of Health Sciences
Department of Chemistry
Toyoake
Aichi 470-1192
Japan

***Jo Lambert***
Ghent University Hospital
Department of Dermatology
De Pintelaan 185
9000 Ghent
Belgium

***Edward J. Land***
Keele University
School of Physical and Geographical Sciences, Lennard-Jones Laboratories
Keele Road
Keele ST5 5BG
UK

***Lionel Larue***
Institut Curie
Developmental Genetics of Melanocytes, INSERM U1021–CNRS UMR3347
Centre Universitaire, 91405 Orsay
France

***Michael S. Marks***
University of Pennsylvania
Departments of Pathology and Laboratory Medicine and Physiology, 513 Stellar-Chance Laboratories
422 Curie Boulevard
Philadelphia, PA 19104-6100
USA

***Wakako Maruyama***
National Research Center for Geriatrics and Gerontology
Department of Cognitive Brain Science
Obu
Aichi 474-8511
Japan

***Makoko Naoi***
Gifu International Institute of Biotechnology
Department of Neurosciences
Kakamigahara
Gifu 504-0838
Japan

***Alessandra Napolitano***
University of Naples "Federico II"
Department of Organic Chemistry and Biochemistry
Via Cinthia 4
80126 Naples
Italy

***M. Concepción Olivares Sánchez***
University of Murcia
Department of Biochemistry and Molecular Biology
Campus de Espinardo
30100 Murcia
Spain

***Stanislav Pavel***
Leiden University Medical Center
Department of Dermatology
PO Box 9600
2300 RC Leiden
The Netherlands
and
Charles University
Department of Dermatology, Faculty of Medicine
Husova 3
306 05 Pilsen
Czech Republic

***Alessandro Pezzella***
University of Naples "Federico II"
Department of Organic Chemistry and Biochemistry
Via Cinthia 4
80126 Naples
Italy

***Mauro Picardo***
San Gallicano Dermatological Institute
Laboratory of Cutaneous Physiopathology
Elio Chianesi 53
00144 Rome
Italy

***Karel Pizinger***
Charles University
Department of Dermatology
Faculty of Medicine
Husova 3
306 05 Pilsen
Czech Republic

***Christopher A. Ramsden***
Keele University
School of Physical and Geographical Sciences, Lennard-Jones Laboratories
Keele Road
Keele ST5 5BG
UK

***Graça Raposo***
Institut Curie
Centre de Recherche
Structure and Membrane Compartments
26 Rue d'Ulm
75248 Paris cedex 05
France

***Peter Riederer***
University of Würzburg
Clinical Neurochemistry, Department of Psychiatry, Psychosomatics and Psychotherapy, and National Parkinson Foundation Centers of Excellence for Neurodegenerative Diseases Research
Füchsleinstrasse 15
97080 Würzburg
Germany

***Patrick A. Riley***
Totteridge Institute for Advanced Studies
The Grange
Grange Avenue
London N20 8AB
UK

***Małgorzata Różanowska***
Cardiff University
School of Optometry and Vision Science
Maindy Road
Cardiff CF24 4LU
UK

***Nico P.M. Smit***
Leiden University Medical Center
Central Laboratory for Clinical Chemistry
Albinusdreef 2
2333 AC Leiden
The Netherlands

***Alain Taïeb***
Hôpital St André
Department of Dermatology and Pediatric Dermatology
CHU de Bordeaux
1 rue Jean Burguet
33077 Bordeaux
France

***Mireille Van Gele***
Ghent University Hospital
Department of Dermatology
De Pintelaan 185
9000 Ghent
Belgium

***Kazumasa Wakamatsu***
Fujita Health University School of Health Sciences
Department of Chemistry
Toyoake
Aichi 470-1192
Japan

# 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), (i) 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 preceeded 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

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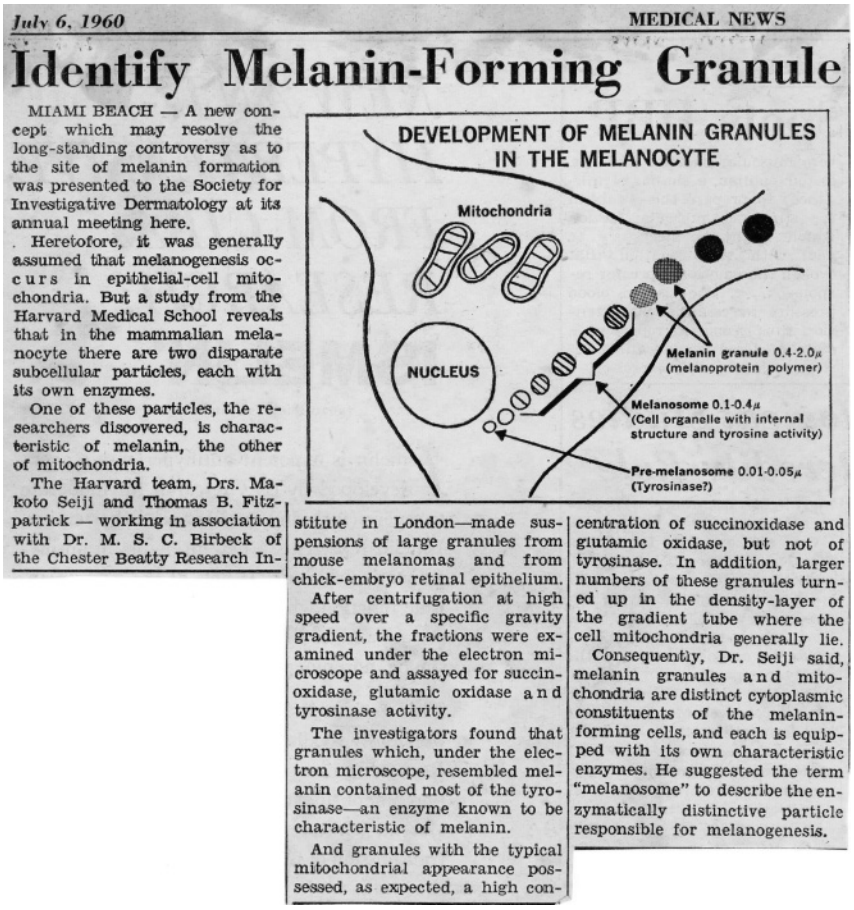

July 6, 1960 MEDICAL NEWS

# Identify Melanin-Forming Granule

MIAMI BEACH — A new concept which may resolve the long-standing controversy as to the site of melanin formation was presented to the Society for Investigative Dermatology at its annual meeting here.

Heretofore, it was generally assumed that melanogenesis occurs in epithelial-cell mitochondria. But a study from the Harvard Medical School reveals that in the mammalian melanocyte there are two disparate subcellular particles, each with its own enzymes.

One of these particles, the researchers discovered, is characteristic of melanin, the other of mitochondria.

The Harvard team, Drs. Makoto Seiji and Thomas B. Fitzpatrick — working in association with Dr. M. S. C. Birbeck of the Chester Beatty Research Institute in London—made suspensions of large granules from mouse melanomas and from chick-embryo retinal epithelium.

After centrifugation at high speed over a specific gravity gradient, the fractions were examined under the electron microscope and assayed for succinoxidase, glutamic oxidase and tyrosinase activity.

The investigators found that granules which, under the electron microscope, resembled melanin contained most of the tyrosinase—an enzyme known to be characteristic of melanin.

And granules with the typical mitochondrial appearance possessed, as expected, a high concentration of succinoxidase and glutamic oxidase, but not of tyrosinase. In addition, larger numbers of these granules turned up in the density-layer of the gradient tube where the cell mitochondria generally lie.

Consequently, Dr. Seiji said, melanin granules and mitochondria are distinct cytoplasmic constituents of the melanin-forming cells, and each is equipped with its own characteristic enzymes. He suggested the term "melanosome" to describe the enzymatically distinctive particle responsible for melanogenesis.

DEVELOPMENT OF MELANIN GRANULES IN THE MELANOCYTE

Mitochondria

NUCLEUS

Melanin granule 0.4-2.0μ (melanoprotein polymer)

Melanosome 0.1-0.4μ (Cell organelle with internal structure and tyrosine activity)

Pre-melanosome 0.01-0.05μ (Tyrosinase?)

**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.

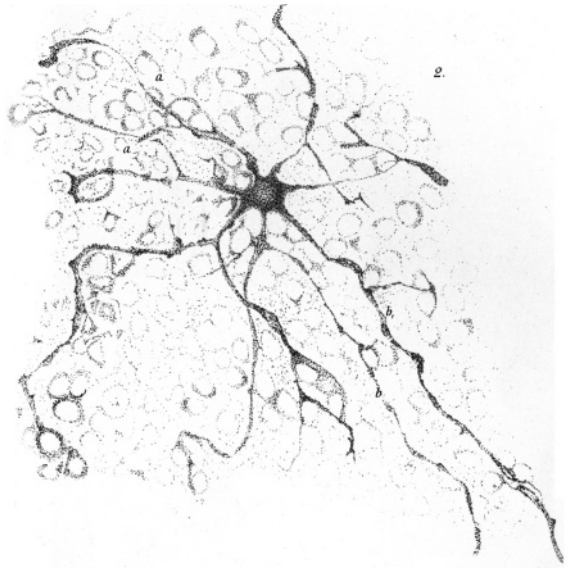

**Figure 1.3** "Chromatophore" from donkey conjuctiva [7].

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

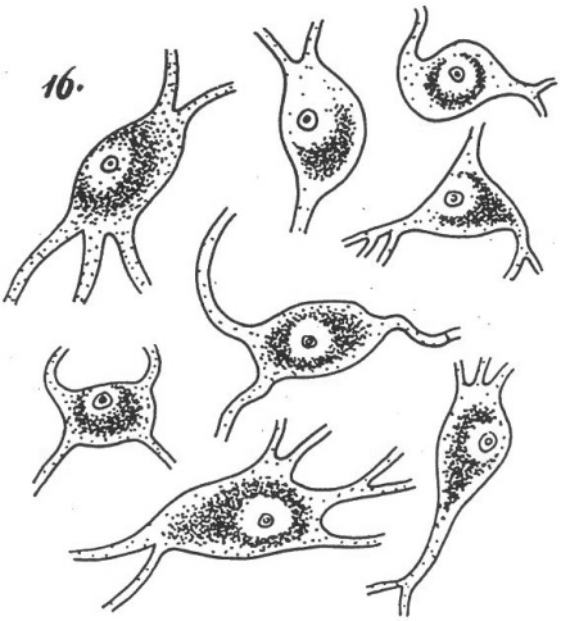


**Figure 1.4** Cells of substantia nigra containing neuromelanin [5].

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 melanoproteins in melanosomes was further confirmed by (i) autoradiographic evidence with [$^{3}$H]dopa and [2-$^{14}$C]dopa [39–43]., (ii) incorporation of [2-$^{14}$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.