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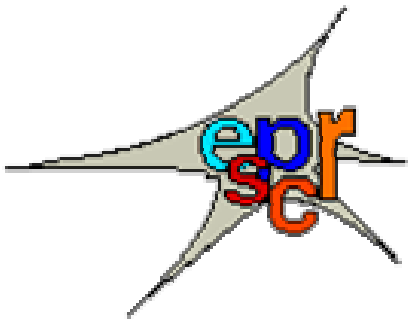

EUROPEAN SOCIETY FOR PIGMENT CELL RESEARCH *BULLETIN*

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HAPPY NEW YEAR 2006

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LETTER TO THE EDITOR
DISCUSSION, REVIEW,
SHORT COMMUNICATION, ...

A Jubilee Year for Two Famous Scientists and Distinguished ESPCR Members

By Jan Borovanský

This year two legends of the ESPCR have reached important anniversaries. They both originated from families with medical connections and graduated as M.D. They both were born in a different country from that where they permanently live nowadays. One of them started his career as a dermatologist to become top scientist in the field of pigment cell research and cell pathology, the other at first was a biochemist interested in melanogenesis and melanogens to become first class dermatologist and photobiologist. They share both a common interest in the toxicity of melanin precursors and a great hobby in performing music. As students they both played in the collegiate jazz bands as a clarinet and alto saxophone and clarinet players, respectively. They share a common weakness for the piano and both of them are my best friends. I introduced them to each other on December 5, 1984.

Professor Patrick Anthony Riley, M.B., B.S., Ph.D., D.Sc., F.I. Biol., F.R.C. Pathol. Was born on 22nd March 1935 in Neuilly-sur-Seine, France. His father Bertrand Hurrell Riley belonged to founders of the World Health Organization and spent many years in Geneva. Patrick Riley visited him regularly during holidays because he studied at a boarding school in the U.K. From childhood he has had a deep fascination for the way things work which can be illustrated e.g. by a set of magnets and one-penny coins he developed much later in his adulthood for a commercial purpose to teach children the phenomenon of magnetism in a playful way. He has always been interested in and fascinated by mechanisms of biological phenomena and his preoccupation in living cells was deepened and „saturated“ by many prominent teachers, scientists and friends, especially at the University College London, which was a powerhouse of biological learning. During medical studies he met his charming wife and life-long supporter, Christine. After graduation from University College Hospital Medical School (UCHMS) in 1960 he spent a half year as a house-physician in the Department of Dermatology and later, having obtained a Rockefeller scholarship, he joined Dr. Arthur Jarrett in the Department of Dermatological Histopathology at UCHMS where he started his research activities by studying various enzyme activities in dendritic epidermal cells. He was awarded a Medical Research Council Junior Clinical Research Fellowship and pursued studies of epidermal depigmentation and the relationship between melanocytes and Langerhans cells. In 1965 he obtained his Ph.D. having defended a thesis entitled Studies of Melanocyte Function and co-authored a textbook of dermatology. In 1966 he was awarded a Beit Memorial Fellowship and moved to the Department of Pathological Chemistry where he shared an office with Trevor Slater, a brilliant and enthusiastic biochemist and advocate of free radical reactions. I suppose that this famous pioneer of free radical research ignited in Patrick Riley his permanent fascination for free radical reactions for in the same year the seminal

paper on photosensitization and lysosomal damage, prepared in cooperation with Professor Trevor Slater, appeared in Nature. In the succeeding period Patrick Riley published a series of depigmentation studies with 4-hydroxyanisole with the aim of utilizing its selective melanocytotoxicity to treat advanced melanoma. After many years research effort this goal was reached: In 1991 a paper describing results of a preliminary clinical trial with hydroxyanisole performed by a surgeon and friend, Brian Morgan, partly confirmed the expectations, although subsequent experience has been disappointing. When Riley joined the Department of Pathological Chemistry (re-named later the Dept. of Chemical Pathology and subsequently the Dept. of Molecular Pathology) it was chaired by Professor Claude Rimington. Here I must repeat a well-known proverb"; The World is small." As a student of medicine I used to work voluntarily (1963-1966) in the laboratory of Professor A.F. Richter who, like Claude Rimington, was interested in porhyrins and in nineteen fifties they had been close pen-friends. In 1970s many of Riley's papers were devoted to the growth of cell populations, to mechanisms of growth inhibition and functional properties of cells in relation to the cell cycle. He was also interested in carcinogenesis and mechanisms of DNA transcription. When the British Council arranged my first meeting with Patrick Riley on March 2, 1978, huge model of double helix was a dominant object in his office. Although our meeting had been planned for half an hour, it lasted a terribly short four hours because our mutual discussion not only revealed plenty of common scientific interests but also very similar „blood group". After 27 years since that meeting I can confirm that it was one of decisive moments of my life which influenced me for ever. Thanks to the British Council Bursary in 1980 and British Council support for Cooperation in Higher Education in 1984 (catalysed by Patrick) I could spend several months in his lab at the UCL in University Street and in Wellington Arms (a discussion pub) just opposite the Dept. of Biochemical Pathology building, studying the effect of zinc and other cations on cultured melanoma and other cells which led to the discovery of the remarkably high zinc cytotoxicity *in vitro.* Since that time I have been able to watch closely the progress of scientific activities in his lab. In 1980s Patrick (with M.Hola) used time-lapse cinematography to study mitoses in cell cultures and cell genealogy. Later the National Foundation for Cancer Research provided his lab with image analysis equipment and Patrick (with D. Spargo) set about trying to devise a quantitative index of cell shape that could be used to generate parameters that could be exploited as diagnostic criteria. In 1985 Patrick Riley became professor at the University College and delivered an inaugural lecture "Pathological Migration: >From Melanin to Malignancy".

In 1991 we published together a paper which emphasized the role of leakage of toxic melanin precursors from aberrant melanosomes in cytotoxic phenomena in melanoma cells. Patrick´s studies, together with S. Naish and C.J. Cooksey, revealed participation of quinones, semiquinones and other radicals in the toxic action of 4-hydroxyanisole. In cooperation with C.J. Cooksey, Stan Pavel, Nico Smit and others, Patrick continued with studies of the cytotoxicity of a series of substituted phenols towards cultured melanoma cells and formulated melanogenesis-targeted antimelanoma therapy and, together with A.M. Jordan and others, the melanocyte-directed prodrug strategy. In 1993 an international research organization devoted to the quinone chemistry - The Quintox Group - was set up (with Patrick Riley as its first chairman and with E.J.Land , C.A.Ramsden and C.J.Cooksey as the most active members) which conducted a range of important studies of melanin biogenesis leading e.g. to rearrangement of first reactions in Raper-Mason scheme. After his retirement in 2000 Patrick did not finish his scientific career; on the contrary he founded Totteridge Institute for Advanced Studies and as *spiritus movens* he has continued to stimulate the research activities of the Quintox Group.

Prof Patrick Riley deserves special thanks for what he had done for the European Society for Pigment Cell Research. Together with G. Prota and N. Cascinelli he founded the Society and acted as its secretary for many years. Since 1995 he has been an honorary member of the ESPCR.

When I was a Ph.D. student and young teacher, there was no internet and I used to visit the Central Medical Library in Prague to search for what´s new in the melanin/melanosome field in the Current Contents regularly on Friday afternoon. At that occasion I came across Patrick Riley´s name. His University College London affiliation induced an imagination of an honorable traditional old

monumental building with very serious, perhaps stiff, scientists in my mind. My very first meeting with Patrick immediately convinced me how wrong I had been. Patrick is typical gentleman constantly radiating English humour around him. He is a charming companion, a „fountain“ of encyclopedic knowledge, an excellent speaker and kind friend. Nothing is too much trouble for him in supporting others. It is no surprise that, at scientific meetings, he is always surrounded by many people longing to speak to him. He influenced me not only scientifically but also in other respects. Thanks to him I could repeatedly listen to discourses at the Royal Institution; he introduced me to London cultural life and, in a period when a harsh regime existed in my country, he kept me saturated with forbidden Solzenicyn novels.

Assoc. Prof. Stanislav Pavel, M.D., Ph.D., Ph.D. was born on July 4, 1945 in Prague, Czechoslovakia. In his younger days he was excellent student and passionate skier (this hobby has survived till present time and his young colleagues have problems to cope with his speed even now). He was vacillating whether to study chemistry, medicine or at the academy of music. In 1963 he decided on medicine (probably under the influence of the family milieu). From the second year of his medical studies at the Faculty of General Medicine (1st Faculty of Medicine), Charles University, Prague he joined students´research activities in the field of melanogens and biochemical markers of melanoma at the 2nd Institute of Medical Chemistry and Biochemistry (renamed later Dept. of Biochemistry and Experimental Oncology). Having graduated in 1969 he became postgraduate student supervised by Professor J. Duchon M.D., PhD. and continued in his research. At the same time he was involved in teaching medical biochemistry. Together with the neurologist, Prof. J. Tichy, M.D., Ph.D., he founded the Medical Dixieland band which is well-known to the pigment cell community from its concerts on the occasions of the the ESPCR Meeting in Amsterdam (1991) and the IPCC in Egmont aan See (2003).

In 1979 he defended his Ph.D. Thesis „Melanogenesis in hamsters with transplanted melanomas“ and in 1980 he submitted his Habilitation Thesis that he could not defend: In the summer of 1981 he was forced to defect to the Netherlands in order to have a clean slate because the secret police had repeatedly pressed him to become an informer. He settled in Groningen, where he had been on a scientific visit couple years before, and continued his studies concerning metabolic manifestations of melanogenesis. He was the first to prove the presence of 5,6-dihydroxyindole in biological material .He demonstrated that melanoma cells excreted O-methylated derivatives of 5,6-dihydroxyindole and determined the chemical structure of conjugates of 5,6-dihydroxyindole and its O-methylated derivatives isolated from urine. In 1986 he moved to Amsterdam and was enrolled into specialization program at the Department of Dermatovenerology to become a clinical dermatologist. In 1988 at the University of Amsterdam he defended his second PhD thesis „Eumelanin-related compounds, their metabolism and clinical relevance“. In January 1992 he joined Department of Dermatology at the University of Leiden as a head of the phototherapy section and director of the Ward section and responsible for allergology. A year later his coworker, Dr Nico Smit, followed him to Leiden and intensive pigment cell research was launched supported in all the respects by the head of Dermatological Department of that time Prof. Bert-Jan Vermeer (distant descendant of famous Dutch painter Vermeer). I was lucky to meet this charming man during my 1993 winter stay in Leiden (and many times after that), when we and friends from Dept. of Electron Microscopy studied melanosome architecture in tyrosinase-transfected fibroblasts where melanogenesis takes place in the absence of melanosomal structural proteins in lysosomes. In Leiden, Dr Pavel has been widely engaged in dermatological practice, in teaching both medical and postgraduate students and has been in charge of many national and international grant projects. He has been involved in much of the research leading to a better understanding of melanogenesis as a potentially toxic process. Nowadays Dr Pavel belongs amongst prominent Dutch dermatologists with deep chemical and biochemical backgrounds. His chemical interest has been further deepened by active research and debates in the Quintox group.

The Pigment Cell Community knows Dr Pavel from his activities in the European Society for Pigment Cell Research Council, in which he was secretary 1994-1997, President 1997-2000 and also Vice-President of the Federation of Pigment Cell Societies.

I shared an office in Prague with Stan for many years. As we had children of similar age, we used to take them regularly during the summer holidays to spend some time in a small village in the hilly southernmost part of Bohemia (leaving our wives in Prague) where, with a glass of red wine in front of a log fire, we had long discussions about the beauties of life. I greatly miss his presence in our department, because he has a gregarious nature with a thoughtful attitude to all his colleagues and students.

I wish Patrick and Stan many more years of productive work and continued good health and happiness both in their professional and personal life.

Message from the IFPCS President to ESPCR Members

Dear friends, members of the ESPCR,

I am greatly honored to serve as the President of the IFPCS, and I look forward for the next three years of leading our federation towards bigger successes and further recognition in the world of scientific research. I begin by expressing my gratitude to the leadership and tremendous efforts of the Past-President, Professor Dot Bennett, who invested so much of her time and energy towards strengthening the ties of the various societies, and facilitating many of the activities of the IFPCS, and to Professor Yasu Tomita for his great service as IFPCS Secretary-Treasurer.

I wish to welcome to the IFPCS the new sister-society, the ASPCR, who undoubtedly will add a new and important dimension to our federation. Vitiligo is a disease that is as challenging to ASPCR scientists and clinicians as melanoma is to those of us in Western countries. The more we learn about the melanocyte, the more knowledge we gain about these two extreme diseases that inflict millions around the world. I hope all of us will continue to appreciate the importance of collaborations, and extend our collaborations to ASPCR members. I am a firm believer that collaborations are synergistic, and the whole is always greater than the sum of its parts. In a world when research funding is challenging, yet means of communication are so easy, it only makes sense to interact scientifically and share resources.

Attending the IPCC, in September 2005, was quite gratifying, not only by the excellent research presented, but also by witnessing how the IFPCS has made us a community, a large international family with close-knit ties. Witnessing the ease of communication and the delight of meeting each other made the IPCC more like a family reunion, without jeopardizing its high professional and scientific standards. We are all indebted to Dr. Vince Hearing for his outstanding organization of such a world-class meeting. But, the success of the meeting would not have been achieved without your attendance and participation. Here, I want to emphasize a very important point, an attitude that prevails in all of the Pigment Cell Research Societies, and transcends to the IFPCS, namely the participation of young scientists, students, postdoctoral fellows and junior faculty members. We always strive to give them a podium, and a chance to present their work at our meetings by providing travel awards, and perhaps equally, or more importantly, a friendly and supportive audience. Many of us, including myself, still fondly remember attending our first IPCC, personally meeting leaders of our field of research, who served as our role models. I sincerely hope that you take it as a special responsibility to introduce young scientists in your laboratories to the communities of ESPCR and IFPCS, by encouraging them to join and become active members. The young scientists of today are the leaders of our specialty in the foreseen future. Let's invest in the future of our young scientists to insure the continuity of pigment cell research.

We should all be proud of Pigment Cell Research, the official journal of the IFPCS. Thanks to the dedication of the Past Editor-in-Chief, Vince Hearing, and the efforts of the current Editor-in-Chief, Colin Goding, we have a very highly respected journal with an impact factor of 3.00. I urge you to make a strong commitment to publish in PCR. The continued success of the journal depends on each one of us. Special thanks go to Colin Goding for giving PCR a new refreshing look, and insuring the expedited review of manuscripts.

Lastly, I want to remind you of acknowledging the importance of research specialty and our model, the pigment cell. It is gratifying to see prominent laboratories, not traditionally known or affiliated with the IFPCS, conducting first class research on the melanocyte. We need to reach out for

such scientists and recruit them to our pigment cell community. By strengthening the individual Pigment Cell Societies, in your case ESPCR, you will ultimately be contributing to the strength of the IFPCS.

I wish you a very happy holiday season and a successful and healthy 2006.

Respectfully yours,

Zalfa Abdel-Malek
IFPCS President

CURRENT LITERATURE

1. Chemistry of Melanins and other Pigments

(Dr. A. Napolitano)

The free radical chemistry of melanin of human retinal pigment epithelium (RPE) and its reactivity under aerobic and anaerobic conditions was investigated in detail by time resolved ESR (Seagle *et al. J Am. Chem. Soc.*). Only one of the different free radical species reacted efficiently with reactive oxygen species, and this was observed with either intrinsic semiquinone-like radicals or extrinsic photogenerated semiquinone radicals. This study confirms the proposed protective role of RPE by melanin from toxic species in both the light and dark.
ESR spectroscopy was also employed: a) to characterize the splenic eumelanin of black C57BL/6 mice and evidencing difference of this pigment from the hair and skin melanin (Plonka *et al*, *Acta Biochim. Pol.*) and b) to study the effect of pH on paramagnetic centers in the pigmented soil fungi Cladosporium cladosporioides (Pilawa, B. *et al.*, *Acta Phys. Pol*).
The reactivity of another fungal melanin from Aspergillus nidulans against hydrogen peroxide and hypochlorous acid was examined in comparison with the standard antioxidant 5-thio-2-nitrobenzoic acid (De Cassia R. Goncalves and Pombeiro-Sponchado, *Biol. Pharm. Bull*). Because its marked ability as hypochlorous acid scavenger this pigment was taken as a promising material for cosmetic industry.
A large number of studies have focused on the inhibition/stimulation of tyrosinase activity and melanin formation by several compounds of the shikimate pathway from various plant sources. Thus extracts from Umbelliferae showed a potent stimulatory effect on melanogenesis with significant enhancement of cell proliferation (Matsuda *et al. Biol.Pharm Bull*). Linear and angular furocoumarins proved the most active. Structure-activity relationship indicated that H or methoxy substituents at the 5 or 8 positions was a requisite for the melanogenesis stimulation activity. Among inhibitors of tyrosinase activity procyanidins trimers and pentamers from apple (Toshihiko *et al. J. Agr. Food. Chem.*) and flavones from Glycyrrhiza uralensis (Kim *et al*, *Planta Medica*) were reported. The compounds responsible for the strong melanin synthesis inhibitory activity were identified and are of potential interest as skin whitening agents. A dihydroderivative of chlorophorin from Chlorophora excelsa was found to be more stable to light than the parent compound and exhibited higher inhibitory effects and lower toxicity toward B16 melanoma cells (Arung *et al*., *Holzforschung*).
A systematic investigation of the structural requirements for the inhibitory effects was carried out on a series of chalcones showing the importance of the 2,4 pattern of hydroxylation on the B ring of these compounds for the highest inhibitory potency (Khatib *et al. Bioorg. Med. Chem.*).

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2. Biology of pigment cells and pigmentary disorders

(Dr. M. Picardo)

The actual update aims to focus on basic melanocyte biology (and in vitro manipulation) and on the vitiligo/melanoma dichotomy.

As regards the basic biology of the melanocytes some relevant papers, proposing monolayer or tridimensional models, should be considered. **Hachiya** provides a tridimensional model of the skin, the human skin substitute (HSS) with melanocytes spontaneously sorted to the basal layer. In this HSS model a physiological, melanocyte phototype-dependent pigmentation takes place. Moreover, as indicated by RT-PCR, SCF/MITF/MAPK/ET1 signal pathway occurs. Consistent with these findings the authors propose this HSS as a useful approach to study hyper- and hypo-pigmentation disorders. Finally, the HSS could help to evaluate the melanocyte stem-like cells. In these last months a lot of papers provide further information about MITF. **Miller**, using molecular biology approaches, focusing on the significance of the post-translational SUMO modifications, indicating that MITF have two conserved sumoylation consensus sites and interacts with two components of the sumoylation pathway (UBC9 and PIAS3). The results of it's study suggest that sumoylation affects MITF activity only when multiple E-boxes are present on MITF targets. **Bordogna** describes development and execution of a functional screen on melanocytes aiming to identify genes involved in the biology of the pigment cells. He reports that Emx1/2 factors down-regulates MITF transcription and induce a de-differentiation process. A downstream target of Emx2 is Wnt1 which could be the mediator of MITF inhibition. MITF, as indicated by **Murakami**, also regulates the expression of the plasminogen activator involved in the remodelling of the extracellular matric and thus promoting the cell invasion. Finally, it is probably irrelevant to mention: the intriguing in vitro study on MITF of **Bismuth**.

A new finding on basic biology of the melanocyte from the Gilchrest team is provided. Indeed, **Kim** by means of WB demonstrates that melanocytes are able to express the receptors for VEGF, depending also on culture conditions. The biological significance remains to be clarified as there isno proliferation, morphological changes, or melanin production. **Krengel** investigated the relatioship between UVB exposure and expression of melanocyte-adhesion molecules, particularly 6-integrin. An interesting overview of the biological significance of the cadherin-mediated adhesion is proposed by **Lilien & Balsamo** and by **Junghans**.

The studies on vitiligo pathogenesis appear to assign a relevant role to the genetic backround both in immunologic and redox status alterations.

Miyamura generated a database based on 200 alleles of the tyrosinase gene from normally pigmented Indian and Japanese subjects. The database was created by sequencing and analysis of 5 exons and a polymorphism study on promoter and exons. The authors describe 12 polymorphisms (11 are racial-dependent) and suggest that the database could help to confirm new mutations easily by checking this database.

Kemp reports that 1858T allele in PTPN22 gene is over-represented in vitiligo patients and PTPN22 encodes the lymphoid protein tyrosine phosphatase already involved in some autoimmune disorders. The same authors (**Akhtar S**) also evaluate the polymorphism in ACE gene but they did not find any significant difference in the frequency of insertion/deletion of a 287-base pair repetitive sequence in intron 16 in contrast with that reported for vitiligo associated with autoimmune disease. This one and another previous discrepancy between the results obtained by different genetic studies, underline a polyfactorial pathogenesis as an univocal positivity for a defined set of gene polymorphisms cannot be indicated for the world vitiligo population. The observation appears to be in agreement with the idea of concomitant genetic, metabolic and external factors which may lead or not to the loss of melanocyte depending on the reciprocal balance. The review of **Zhang** and coworkers on the possible genetic background for vitiligo represents a helpful guide even for non geneticists as it provides an overview on the actual approaches.

Jadali, starting from the idea of a prevalent immunological pathogenesis, performed a random peptide library for the determination of antibody specificity found in the serum of vitiligo patients. However, the results obtained appear to be too distant from the defintion of a pathogenetic role. **Schallreuter** provides a new piece in the puzzle of vitiligo. She shows that in vitiligo patients there is an altered

calcium-dependent uptake of phenylalanine, both in vivo and in vitro, thus affecting the initial steps of the melanogenesis.
Now, from the pathogenesis to the treatment. Tacrolimus has been succesfully used for the treatment of the vitiligo but how it works and why? **Lan** observes like in vitro, tacrolimus is able to act directly on keratinocytes by inducing CSF and MMP2 release. Through this mechanism it promotes melanocyte growth and proliferation and it could represent the cellular basis for the effectiveness in the treatment of the vitiligo. **Falabella** proposes an overview of the actual surgical methods and remarks that the appropriate selection of the patients is necessary to achieve the best results. **Mulekar** reports a 6-year follow-up of 142 patients treated with autologous non-cultured melanocyte/keratinocyte transplantation. He shows that the transplantation of a fresh non-cultured suspension of melanocyte/keratinocyte can give good repigmentation but the result obtained is independent of previous repigmentation in another area or test graft. It is thus possible that the disease can be active even if the lesions are apparently stable. The suggestion of Mulekar is in agreement with the idea of an intrinsic melanocyte defect which is more or less clinically evident. However, until now the panel of methods used for melanocyte or melanocyte/keratinocyte isolation is wide and the culture for in vitro or in vivo study is not univocal and obvious. **Lin** suggests the utilization of chitosan-coated membranes to improve the growth of the melanocytes. The chitosan-induced spheroid morphology does not affect the phenotype of the melanocytes that are able to return to the normal dendrititc morphology after the reinoculation on collagen-coated surface. The method could be useful both for preliminary culture for cell transplantation in vitiligo patients and in vitro culture of melanocytes from vitiligo subjects. **Hartmann** performed a trial vs placebo (four-quarters comparison) for the evaluation of the effectiveness of a combinatory therapy narrow-band UVB plus topical calcipotriol. In agreement with current literature, the authors report a higher effectiveness of the narrow-band UVB compared with broad-band UVB but they cannot indicate an improvement of UVB therapy by the topical application of calcipotriol.
A lot of in vivo and in vitro evidence suggest that melanoma and vitiligo can be considered the opposite face of the same phenomenon. The understanding of vitiligo pathogenesis could represent a lesson for the approaches to the melanoma. **Palermo** hypothesizes that if in both diseases there is an abnormal (different degree of avidity) immune response against the same antigen, it is possible to utilize the peripheral cytotoxic melanocyte-specific TCD8 cells from vitiligo subjects for the treatment of melanoma. Obviously, a HLA compatiblity between vitiligo and melanoma patients is the crucial requisite,. The results of the in vivo trial could open a new scenario for melanoma treatment in the near future. Regarding melanoma: **Tonks** demonstrates a different role in vivo and in vitro for pocket proteins during proliferation of the melanocytes and progression of melanoma. A further update on the role of a-MSH and ET-1 was provided by **Kadekaro**. She indicates a possible intracellular pathway involving a-MSH, MC1R, Akt, CREB and Mitf and counteracting the apoptotic effect of UV. In normal melanocytes, thus, the UV-induced ET-1 production protects the cells from the apoptosis and from mutagenesis (associated with hydrogen peroxide production and DNA damage) whereas loss of function mutations of MC1R increases a risk of melanoma. An *in vitro* study (from a molecular approach) of the role of p53 in melanoma, paying partucular attention to its changes in stability, localization, and activity based on different DNA-damaging agents, represents the aim of the paper of **Razorenova**. Using two different melanoma cell lines, **Mangahas** shows that ET1, through ETB receptor, activates CXCL1 and CXCL8 in melanoma but not in normal melanocyte cell lines. The biological relevance is evident because ET1 downregulates e-cadherin, which inhibits the tumor invasion, and activates metalloproteinases, which promote the migration and invasion processes.
Two papers sorted from PubMed are clinical studies. **Guerra-Tapia** describes, in a young subject, a case of vitiligo in congenital divided nevus evolving in halo nevus. The clinical pattern is interesting and requires, as suggested by the authors, a prolonged follow-up but it is improbable a common pathogenesis for vitiligo and halo nevus. The other paper is that of **Loquai** which describes a case of confetti-like lesions with hyperkeratosis in a 33 year old white man affected by vitiligo and mycosis fungoides. The electron microscopy and the histology corroborate a different entity for the confetti-like spots compared to the white vitiligo lesions in the same subjects.

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3. MSH, MCH, other hormones, differentiation

(Dr. R. Morandini)

Takahashi studied the postranslational processing of proopiomelanocorting family molecules; in another paper the same authors described three proopiomelanocortin subtype genes and their expression in the barfin flounder pituitary. Another interesting paper also from the same authors, presented the « Evolution of Melanocortin systems in fish ».

It is well known that α-MSH is produced through a processing of POMC. Eves at al. made an interesting review on the role of the alpha-Melanocyte stimulating hormone in the inflammation in human melanoma. This review considers the evolving biology of alpha-MSH and discusses its role in man that extend far beyond pigmentation of skin melanocytes, suggesting that the detoxifying role of alpha-MSH in inducing melanogenesis is only one aspect of the stress-coping role of this hormone.

Many studies about the effect of alpha-MSH have been published lately such as:

- Alpha-melanocyte stimulating hormone reduces putative stress-induced sickness behaviors in isolated guinea pig pups by Schiml-Webb;
- Signaling pathways implicated in alpha-melanocyte stimulating hormone-induced lipolysis in 3T3-L1 adipocytes by Cho KJ;
- Effect of alpha-melanocyte-stimulating hormone on interleukin 8 and monocyte chemotactic protein 1 expression in a human retinal pigment epithelial cell line by Cui HS;

It is interesting to point that α-MSH suppresses antigen-induced lymphocyte proliferation in humans independently of melanocortin 1 receptor gene status.
(NDP)-alpha-MSH (α-MSH agonist) have been used to prolong allograft survival in experimental heart transplantation (paper by Colombo). The preliminary results seem to be very promising: the analog preserves heart function through a broad effect on multiple pathways and suggest that the peptide could improve the outcome of organ transplantation in combination with immunosuppressive treatments. These effects can be also explained partially because the analog is more stable than the native hormone.

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4. Photobiology

(Dr. N. Smit)

In a letter to the editor Hacker et al describe the various mouse models and genetic backgrounds that are capable of producing UVR induced melanoma. They used a brown mouse strain with a melanocyte-specific Hras mutant (G12V) transgene (TPras). The animals received a neonatal regimen of UVR (with Philips FS40 UVB lamps) and obtained cutaneous melanoma tumors with high penetrance (57%) whereas previous chronic exposure regimen did not result in development of tumors. There was a marked reduction in pigmentation of the neonatal TPras animals as compared to the adult highly pigmented TPras animals. The UV induced melanoma formation did not always involve loss of Ink4A or Arf and the authors concluded that NRAS and BRAF mutations co-operate with solar UVR in development of melanoma.
Induction of melanoma in the different mouse models strongly depends on the genetic background and also on the pigmentation characteristics. Chintala et al describe the subtle gray (sut) mouse where a mutation in the Slc7a11 gene is responsible for a low rate of extracellular cystine transport. Next to influences on pheomelanin pigmentation the reduced cystine transport is also critical for cell proliferation and protection from oxidative stress. The sut mice are considered as a model for oxidative stress-related diseases. It could be possible that a strong uptake of cystine by pheomelanogenesis may also reflect such a situation in some cases of melanocytes with RedHairColour phenotype. The paper by Corre et al reviews the role of the upstream stimulating factors (USF). In melanocytes USF-1 has been implicated to play an important role in the (UV)-activation of genes associated with pigmentation. Next to the role of USF-1 in the tanning response the place of USF-transcription factors in various regulatory networks is outlined in the paper. In the introduction of the paper by Marrot et al also the induction of melanoma tumors in mouse models (see above) is mentioned and the lack of a clear "UV signature" in the melanoma cells is discussed. Various stress factors were already implicated in gene expression studies performed by others after UVA irradiation of melanocytes. In the study by Marrot induction of important cell cycling regulating proteins such as p21 and GADD45 were shown at various time points after different doses of solar simulated radiation (both UVB and UVA). A 10 min UV irradiation induced both p21 and GADD45 within 4 hrs. Twenty min irradiation resulted in a more prominent induction of GADD45 lasting up to 15 hrs whereas the p21 response was delayed (24 hrs). Effects on cell cycle and accumulation of cells in G2-M phase were shown by FACS analysis that may correlate well with the GADD45 induction. Another new finding is the early induction of heme oxygenase 1(HO-1) by SSR and UVA in the melanocytes. Interestingly this HO-1 induction was increased when melanogenesis in the cells was stimulated. This may be another important indication that intracellular melanin plays a role in UV induced stress.
The paper by Curtin et al offers an interesting contribution to the discussion about melanoma, the role of UV-B or UV-A induced damage, pigmentation and "stress" cell cycling regulatory pathways. As was shown earlier in one of the refs (16 Maldonado et al JNCI 2003) BRAF and N-RAS mutations on skin without chronic sun-induced damage (non-CSD) occurred in 81% of melanomas. In other types (CSD, acral and mucosal) of melanoma a majority had mutations in neither gene. Among the four groups significant differences in copy numbers of CDKN2A, PTEN, CDK4 and CCND1 were demonstrated. Changes in the MAP-Kinase and PI3Kinase pathways are considered to be of major importance in melanoma. Next to BRAF and N-RAS, CDK4 and CCND1 could act as independent oncogenes promoting proliferation while loss of PTEN may influence survival via the PI3Kinase pathway.

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5. Neuromelanins

(Prof. M. d'Ischia)

In the last months of 2005 several papers addressed important issues concerning biochemical changes in dopaminergic systems and the structure and function of neuromelanin and associated proteins, as well as the definition of appropriate model systems to study pigmented dopaminergic neurones.
Purisai et al. (2005) assessed the relationship between toxic injury and α-synuclein expression in the substantia nigra of squirrel monkeys treated with a single injection of MPTP. The results indicate a sustained α-synuclein up-regulation in the primate brain, supporting a direct relationship between neuronal injury and enhanced α-synuclein expression. An attractive possibility is that protein elevation within cell bodies may be a late feature of neurons under toxic stress.
An interesting and critical study by Li et al. (2005) examined on a comparative basis the effects of neuromelanin (NM) from the human substantia nigra and synthetic dopamine melanin (DAM) on neuronal and glial cell lines and on primary rat mesencephalic cultures. Analysis of various parameters was suggestive of different protective roles for NM and DAM under conditions of high oxidative load, which would require clarification of the main structural differences between the natural and synthetic pigment. On this basis, the authors suggested a caveat about interpretation of pathogenetic mechanisms based only upon use of DAM as a model system. The issue of biological model systems for pigmented dopaminergic neurones was also addressed by Oestergren, et al. (2005) who proposed dopamine melanin loaded-PC12 cells.
Following an early observation that BH4 and DA cause oxidative damage to dopaminergic cells, Kim et al. (2005) investigated the effect of immobilization stress on the nigrostriatal system and found an elevation in BH4 and DA levels as well as in their synthesizing enzymes, tyrosine hydroxylase and GTP cyclohydrolase I. This finding suggests that a severe stress can cause oxidative damage to the DA neurons in vivo, with possible relevance to Parkinson's disease.
Proteomic analysis of intact isolated neuromelanin granules by Tribl et al. (2005) indicated the presence of numerous proteins derived from lysosomes or lysosome-related organelles originating from the endosome-lysosome lineage. In addition, endoplasmic reticulum-derived chaperones were identified, including the transmembrane protein calnexin, which has recently been located in lysosome-related melanosomes, and has been suggested to be a melanogenic chaperone.
A critical review of the numerous and various. functions so far attributed to neuromelanin and an attempt to provide a unified theory based on the physical and chemical properties of the black particle (the neuromelanin cage) was offered by Bruno Nicolaus (2005).
Finally, a review on the development and aging of neuromelanin (NM)-containing neurons in the central nervous system was offered by Itzev et al. (2005).

- Itzev, Dimitar E.; Ovtscharoff, Wladimir A.; Marani, Enrico; Usunoff, Kamen G.
  Neuromelanin-containing, catecholaminergic neurons in the human brain: ontogenetic aspects, development and aging. Biomedical Reviews 13:39-47, 2002
  Abstract: A review. The authors discuss data on the development and aging of neuromelanin (NM)-contg. neurons in the central nervous system. Neuromelanin is brownish-to-black pigment that accumulates in the catecholaminergic (noradrenergic and dopaminergic) neurons and is a reliable natural marker that delineates the A1-A14 catecholaminergic groups of Dahlstrom and Fuxe in the human brain. The pigmentation of noradrenergic locus ceruleus neurons starts earlier than that of dopaminergic substantia nigra, but also a considerable individual variability is present. The pigmentation is well advanced in adolescence. The data at what age the maximal pigmentation is reached are controversial, as are the data on the cell loss in the NM-contg. neuronal populations by normal aging. Thus, the participation of NM in the pathogenesis of Parkinson's disease remains enigmatic.

- Kim, Sung Tae; Choi, Ji Hyun; Chang, Jin Woo; Kim, Seong Who; Hwang, Onyou.

Immobilization stress causes increases in tetrahydrobiopterin, dopamine, and neuromelanin and oxidative damage in the nigrostriatal system. Journal of Neurochemistry 95(1): 89-98, 2005.

Abstract : Oxidative stress is believed to contribute to the pathophysiol. of Parkinson's disease, in which nigrostriatal dopaminergic (DA) neurons undergo degeneration. Identification of endogenous mols. that contribute to generation of oxidative stress and vulnerability of these cells is crit. in understanding the etiol. of this disease. Exposure to tetrahydrobiopterin (BH4), the obligatory cofactor for DA synthesis, was obsd. previously to cause oxidative damage in DA cells. To demonstrate the physiol. relevance of this observation, we investigated whether an overprodn. of BH4 and DA might actually occur in vivo, and, if it did, whether this might lead to oxidative damage to the nigrostriatal system. Immobilization stress (IMO) elevated BH4 and DA and their synthesizing enzymes, tyrosine hydroxylase and GTP cyclohydrolase I. This was accompanied by elevation of lipid peroxidn. and protein-bound quinone, and activities of antioxidant enzymes. These increases in the indexes of oxidative stress appeared to be due to increased BH4 synthesis because they were abolished following administration of the BH4 synthesis inhibitor, 2,4-diamino-6-hydroxy-pyrimidine. IMO also caused accumulation of neuromelanin and degeneration of the nigrostriatal system. These results demonstrate that a severe stress can increase BH4 and DA and cause oxidative damages to the DA neurons in vivo, suggesting relevance to Parkinson's disease.

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  Differential effects of human neuromelanin and synthetic dopamine melanin on neuronal and glial cells. Journal of Neurochemistry 95(2): 599-608, 2005.

  Abstract : We investigated the effects of neuromelanin (NM) isolated from the human substantia nigra and synthetic dopamine melanin (DAM) on neuronal and glial cell lines and on primary rat mesencephalic cultures. Lactate dehydrogenase (LDH) activity and lipid peroxidn. were significantly increased in SK-N-SH cells by DAM but not by NM. In contrast, iron-satd. NM significantly increased LDH activity in SK-N-SH cells, compared with 100 mg/mL ETDA-treated NM contg. a low concn. of bound iron. DAM, but not NM, stimulated hydroxyl radical prodn. and increased SK-N-SH cell death via apoptotic-like mechanisms. Neither DAM nor NM induced any changes in the glial cell line U373. 3H-Dopamine uptake in primary rat mesencephalic cultures was significantly reduced in DAM- compared with NM-treated cultures, accompanied by increased cell death via an apoptosis-like mechanism. Interestingly, Fenton-induced cell death was significantly decreased in cultures treated with both Fenton reagent and NM, an effect not seen in cultures treated with Fenton reagent plus DAM. These data are suggestive of a protective role for neuromelanin under conditions of high oxidative load. Our findings provide new evidence for a physiol. role for neuromelanin in vivo and highlights the caution with which data based upon model systems should be interpreted.

- Nicolaus, Bruno J. R.

  A critical review of the function of neuromelanin and an attempt to provide a unified theory. Medical Hypotheses 65(4): 791-796, 2005.

  Abstract : This paper provides a crit. review of the numerous and various biol. functions so far attributed to neuromelanin and an attempt to provide a unified theory based on the peculiar phys. and chem. properties of the black particle (the neuromelanin cage). It is stressed that neuromelanin is not homogeneous, as is commonly accepted, but is made up of different substrate specific black pigments formed by the oxidn. of o.diphenols or other oxygenated precursors (substantia nigra melanin, locus coeruleus melanin, retinal pigmented epithelium or ocular melanin, inner-ear melanin, and so on). Ocular melanin is believed to protect the eye by trapping metals and free radicals. The paper shows that this unconfirmed mechanism is a rather fortuitous irreversible mol. accident, which at times may prove itself deleterious. Albinism often leads to deafness in animals, indicating a genetic correlation. These two conditions appear to be correlated at a mol. level to

eye/ear pigmentation and suggest verifying this hypothesis in normal and albino human individuals. Skin and ocular melanin are chem. different. However, they are both involved in light absorption/dissipation. The black particle structure (melanin cage) is believed to be fundamental to this process because there is a common bioelec. mechanism. The latter is worth of further investigation. It is also proposed checking how ocular melanin dissipates the excessive absorbed light (as heat or as current). It has been claimed that inner-ear melanin mutes acoustic waves. This paper suggests investigating the underlying mechanism and also studying whether this pigment is bio-elec. involved in audiol. According to numerous authors, substantia nigra melanin is only biol. garbage. This view is rejected, and it is stressed that intracellular melanogenesis is a fundamental and genetically controlled physiol. process. It has been repeatedly claimed that the binding of iron, heavy metals, free radicals and harmful chems.
By substantia nigra melanin is fundamental to body detoxification/protection. Presumably, such irreversible and generic binding mechanisms have no physiol. foundation; it is suggested the alternative that, substantia nigra melanin acts as semiconductor, transmitting and modulating nervous impulses, in a reversible way. In fact, substantia nigra melanin is absent or significantly scarce in two conditions of life in which the coordination of movement is either inefficient (newborn babies) or strongly compromised (Parkinson). To check this assumption, further investigation of nucleus caudatus, putamen, globus pallidus, substantia nigra pars compacta and reticulata, nucleus hypothalamicus is recommended.

- Oestergren, Anna; Svensson, Anne-Lie; Lindquist, Nils Gunnar; Brittebo, Eva B.
Dopamine melanin-loaded PC12 cells: A model for studies on pigmented neurons. Pigment Cell Research 18(4): 306-314, 2005.
Abstract : The most conspicuous feature in idiopathic parkinsonism is the degeneration of pigmented neurons in the substantia nigra. A major problem for the study of the significance of neuromelanin for the development of parkinsonism is that common exptl. animals lack neuromelanin in substantia nigra. The aim of this study was to develop an in vitro model that could be used to study the role of neuromelanin in chem. induced toxicity in dopaminergic cells. Cultured neuron-like PC12 cells were exposed to synthetic dopamine melanin (0-1.0 mg/mL) for 48 h, resulting in uptake of dopamine melanin particles into the cells. The intracellular distribution of dopamine melanin granules was similar to that found in neuromelanin-contg. neurons. Dopamine melanin, up to 0.5 mg/mL, had negligible effects on ultrastructure, induction of the endoplasmic reticulum-stress protein glucose regulating protein 78, activation of caspase-3 and cell viability. The decreased cell viability in response to the cytotoxic peptide amyloid- 25-35 was similar in melanin-loaded cells and in control cells without melanin. The results of the studies suggest that melanin-loaded PC12 cells can serve as an in vitro model for studies on the role of neuromelanin for the toxicity of chems., in particular neurotoxicants with melanin affinity, in pigmented neurons.

- Purisai, Maya G.; McCormack, Alison L.; Langston, William J.; Johnston, Louisa C.; Di Monte, Donato A.
-Synuclein expression in the substantia nigra of MPTP-lesioned non-human primates. Neurobiology of Disease 20(3): 898-906, 2005.
Abstract : Changes in the expression of -synuclein are likely to underlie its normal function as well as its role in pathol. processes. The relationship between toxic injury and -synuclein expression was assessed in the substantia nigra of squirrel monkeys treated with a single injection of MPTP and sacrificed 1 wk or 1 mo later. At 1 wk, when stereol. cell counting revealed only a small decrease (-10%) in the no. of dopaminergic neurons, -synuclein mRNA and protein were markedly enhanced. Increased -synuclein immunoreactivity was evident at the level of neuronal fibers whereas nigral cell bodies were devoid of detectable protein. At 1 mo post-MPTP, neuronal loss rose to 40%. Both -synuclein mRNA and protein remained elevated but, noticeably, a robust -synuclein immunoreactivity characterized a significant no. of cell bodies. Neuromelanin granules are hallmarks of dopaminergic neurons in primates. Therefore, the no. of -synuclein-

pos. cells that also contained neuromelanin was counted throughout the substantia nigra. At 1 mo, the vast majority of -synuclein-immunoreactive neurons contained neuromelanin, and approx. 80% of the dopaminergic cell bodies that survived MPTP toxicity stained pos. for -synuclein. The results indicate that a single toxic insult is capable of inducing a sustained -synuclein up-regulation in the primate brain. They support a direct relationship between neuronal injury and enhanced -synuclein expression, and suggest that protein elevation within cell bodies may be a late feature of neurons that have endured a toxic stress.

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"Subcellular proteomics" of neuromelanin granules isolated from the human brain. Molecular and Cellular Proteomics 4(7), 945-957, 2005.
Abstract : "Subcellular proteomics" is currently the most effective approach to characterize subcellular compartments. Based on the powerful combination of subcellular fractionation and protein identification by LC-MS/MS, we were able for the first time to (1) isolate intact neuromelanin granules from the human brain and (2) establish the first protein profile of these granules. This compartment contg. neuromelanin (NM) is primarily located in the primate's substantia nigra, one of the main brain regions that severely degenerates in Parkinson's disease. We used mech. tissue disaggregation, discontinuous sucrose gradient centrifugation, cell disruption, and organelle sepn. to isolate NM granules from human substantia nigra. Using transmission electron microscopy, we demonstrated that the morphol. characteristics of the isolated NM granules are similar to those described in human brain tissue. We found numerous proteins demonstrating a close relationship of NM-contg. granules with lysosomes or lysosome-related organelles originating from the endosome-lysosome lineage. Intriguingly, we further revealed the presence of endoplasmic reticulum-derived chaperones, esp. the transmembrane protein calnexin, which recently has been located in lysosome-related melanosomes, and has been suggested to be a melanogenic chaperone.

6. Genetics, molecular and developmental biology

(Dr. F. Beermann)

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Abstract: The aggressive clinical behavior of melanoma suggests that the developmental origins of melanocytes in the neural crest might be relevant to their metastatic propensity. Here we show that primary human melanocytes, transformed using a specific set of introduced genes, form melanomas that frequently metastasize to multiple secondary sites, whereas human fibroblasts and epithelial cells transformed using an identical set of genes generate primary tumors that rarely do so. Notably, these melanomas have a metastasis spectrum similar to that observed in humans with melanoma. These observations indicate that part of the metastatic proclivity of melanoma is attributable to lineage-specific factors expressed in melanocytes and not in other cell types analyzed. Analysis of microarray data from human nevi shows that the expression pattern of Slug, a master regulator of neural crest cell specification and migration, correlates with those of other genes that are important for neural crest cell migrations during development. Moreover, Slug is required for the metastasis of the transformed melanoma cells. These findings indicate that melanocyte-specific factors present before neoplastic transformation can have a pivotal role in governing melanoma progression.

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To determine the effects of bHLH factor expression on pigment cell development, the neurogenic bHLH factor Mash1 was expressed early in pigment cell development in transgenic mice from the dopachrome tautomerase (Dct) promoter. Dct:Mash1 transgenic mice exhibit microphthalmia and coat color hypopigmentation. Mash1 expression in the retinal pigmented epithelium (RPE) initiates neurogenesis in this cell layer, whereas expression in remaining neural crest-derived melanocytes alters their differentiation, in part by profoundly downregulating expression of the p (pink-eyed dilution) gene, while maintaining their cell fate. The effects of transcriptional perturbation of pigment cell precursors by Mash1 further highlight differences between pigment cells of distinct developmental origins, and suggest a mechanism for the alteration of melanogenesis to result in marked coat color dilution.

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Abstract: Microphthalmia-associated transcription factor (MITF) M-form is a melanocyte-specific transcription factor that plays a key role in melanocyte development, survival, and differentiation. Here, we identified MITF as a new substrate of caspases and we characterized the cleavage site after Asp 345 in the C-terminal domain. We show that expression of a noncleavable form of MITF renders melanoma cells resistant to apoptotic stimuli, and we found that the C-terminal fragment generated upon caspase cleavage is endowed with a proapoptotic activity that sensitizes melanoma

cells to death signals. The proapoptotic function gained by MITF following its processing by caspases provides a tissue-restricted means to modulate death in melanocyte and melanoma cells.

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Summary: Mice with a conditional knockout of Rb were genereated using Tyr::Cre mice and "floxed" Rb mice. These mice had no apparent phenotype, and melanocyte morphology and number seemed normal. In contrast, Rb-deficient melanocytes cultured in vitro showed increased proliferation, and decreased requirement for mitogens.

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Temperature sensitive oculocutaneous albinism associated with missense changes in the tyrosinase gene. Br J Ophthalmol 89(10):1383-1384, 2005.

- Wellbrock C, Marais R.
Elevated expression of MITF counteracts B-RAF-stimulated melanocyte and melanoma cell proliferation. J Cell Biol 170(5):703-708, 2005.

- Yamazaki F, Okamoto H, Matsumura Y, Tanaka K, Kunisada T, Horio T.
Development of a new mouse model (xeroderma pigmentosum a-deficient, stem cell factor-transgenic) of ultraviolet B-induced melanoma. J Invest Dermatol 125(3):521-525, 2005.

7. Tyrosinase, TRPs, other enzymes

(Prof. J.C. Garcia-Borron)

- Ando H, Wen ZM, Kim HY, Valencia JC, Costin GE, Watabe H, Yasumoto KI, Niki Y, Kondoh H, Ichihashi M, Hearing VJ.
  Intracellular composition of fatty acid affects the processing and function of tyrosinase through the ubiquitin-proteasome pathway. Biochem J. 2005 Oct 19; [Epub ahead of print]
  Proteasomes are multicatalytic proteinase complexes within cells that selectively degrade ubiquitinated proteins. We have recently demonstrated that fatty acids, major components of cell membranes, are able to regulate the proteasomal degradation of tyrosinase, a critical enzyme required for melanin biosynthesis, in contrasting manners by relative increases or decreases of the ubiquitinated tyrosinase. In this study, we show that altering the intracellular composition of fatty acids affects the post-Golgi degradation of tyrosinase. Incubation with linoleic acid (C18:2) dramatically changed the fatty acid composition of cultured B16 melanoma cells, i.e. the remarkable increase of polyunsaturated fatty acids such as linoleic acid and arachidonic acid (C20:4) was compensated by the decrease of monounsaturated fatty acids such as oleic acid (C18:1) and palmitoleic acid (C16:1), with little effect on the proportion of saturated to unsaturated fatty acid. When the composition of intracellular fatty acids was altered, tyrosinase was rapidly processed to the Golgi apparatus from the endoplasmic reticulum (ER) and the degradation of tyrosinase was increased after its maturation in the Golgi. Retention of tyrosinase in the ER was observed when cells were treated with linoleic acid in the presence of proteasome inhibitors, explaining why melanin synthesis was decreased in cells treated with linoleic acid and a proteasome inhibitor despite the abrogation of tyrosinase degradation. These results suggest that the intracellular composition of fatty acid affects the processing and function of tyrosinase in connection with the ubiquitin-proteasome pathway and suggest that this might be a common physiologic approach to regulating protein degradation.

- Arakane Y, Muthukrishnan S, Beeman RW, Kanost MR, Kramer KJ.
  Laccase 2 is the phenoloxidase gene required for beetle cuticle tanning. Proc Natl Acad Sci U S A. 102(32):11337-42, 2005. Epub 2005 Aug 2.
  Cuticle tanning (or sclerotization and pigmentation) in invertebrates involves the oxidative conjugation of proteins, which renders them insoluble and hardens and darkens the color of the exoskeleton. Two kinds of phenoloxidases, laccase and tyrosinase, have been proposed to participate in tanning, but proof of the true identity of the enzyme(s) responsible for this process has been elusive. We report the cloning of cDNAs for laccases and tyrosinases from the red flour beetle, Tribolium castaneum, as well as their developmental patterns of expression. To test for the involvement of these types of enzymes in cuticle tanning, we performed RNA interference experiments to decrease the levels of individual phenoloxidases. Normal phenotypes were obtained after dsRNA-mediated transcript depletion for all phenoloxidases tested, with the exception of laccase 2. Insects injected with dsRNA for the laccase 2 gene failed to tan, were soft-bodied and deformed, and subsequently died in a dsRNA dose-dependent fashion. The results presented here support the hypothesis that two isoforms of laccase 2 generated by alternative splicing catalyze larval, pupal, and adult cuticle tanning in Tribolium.

- Arredondo M, Nunez MT.
  Iron and copper metabolism. Mol Aspects Med. 26(4-5):313-27, 2005.
  Iron and copper are essential nutrients, excesses or deficiencies of which cause impaired cellular functions and eventually cell death. The metabolic fates of copper and iron are intimately related. Systemic copper deficiency generates cellular iron deficiency, which in humans results in diminished work capacity, reduced intellectual capacity, diminished growth, alterations in bone mineralization, and diminished immune response. Copper is required for the function of over 30 proteins, including superoxide dismutase, ceruloplasmin, lysyl oxidase, cytochrome c oxidase,

tyrosinase and dopamine-beta-hydroxylase. Iron is similarly required in numerous essential proteins, such as the heme-containing proteins, electron transport chain and microsomal electron transport proteins, and iron-sulfur proteins and enzymes such as ribonucleotide reductase, prolyl hydroxylase phenylalanine hydroxylase, tyrosine hydroxylase and aconitase. The essentiality of iron and copper resides in their capacity to participate in one-electron exchange reactions. However, the same property that makes them essential also generates free radicals that can be seriously deleterious to cells. Thus, these seemingly paradoxical properties of iron and copper demand a concerted regulation of cellular copper and iron levels. Here we review the most salient characteristics of their homeostasis.

- Chaki M, Mukhopadhyay A, Chatterjee S, Das M, Samanta S, Ray K.
Higher prevalence of OCA1 in an ethnic group of eastern India is due to a founder mutation in the tyrosinase gene. Mol Vis. 11:531-4, 2005.
PURPOSE: Oculocutaneous albinism (OCA) is a group of autosomal recessive disorders characterized by deficient synthesis of melanin pigment and associated with common developmental abnormalities of the eye. It is one of the major causes of childhood blindness in India. The disease is common among an ethnic group (Tili) of Eastern India, which represents about 12.56% of the Bankura district population (approximately 0.4 million) of West Bengal. The purpose of the study was to investigate the molecular lesions causing OCA within this ethnic group for the unequivocal diagnosis of the carriers and attempt to decipher the cause for the high prevalence of OCA. METHODS: Fourteen OCA-affected Tili families consisting a total of 161 individuals, including 26 patients, were recruited for the study. A lack of tyrosinase (TYR) activity among all the patients was ascertained by the tyrosinase hair bulb assay. Mutation screening in the tyrosinase gene (TYR) was done by single strand conformational polymorphism (SSCP) and DNA sequencing. The restriction fragment length polymorphism (RFLP) assay was carried out to determine the frequency of the pathogenic changes among the normal individuals. Haplotype analysis was performed at the TYR locus using a set of informative microsatellite and SNP markers. RESULTS: All the patients were homozygous for a null mutation (c.832C>T, Arg278stop) in TYR exon 2, which might cause a complete loss of enzyme activity. The mutation occurred in the same haplotype background. The frequency of the disease in this ethnic group was estimated to be significantly higher than the world average. CONCLUSIONS: OCA1 in the Tili population is due to the occurrence of a founder mutation in the TYR as indicated by haplotype analysis. Higher prevalence of the mutation in the population group is due to marriage within the same community. The diagnostic RFLP assay can be utilizad for genetic counseling and thereby will help to reduce the disease load on the population.

- Chang TS, Ding HY, Lin HC.
Identifying 6,7,4'-trihydroxyisoflavone as a potent tyrosinase inhibitor. Biosci Biotechnol Biochem. 69(10):1999-2001, 2005.
A known biotransformed compound, 6,7,4'-trihydroxyisoflavone, was identified as a potent tyrosinase inhibitor. It inhibited mushroom tyrosinase with an IC(50) value of 9.2 muM, which is six times the anti-tyrosinase activity of kojic acid (IC(50)=54.4 muM). The inhibition kinetics, analyzed by Lineweaver-Burk plots, indicated 6,7,4'-trihydroxyisoflavone to be a competitive inhibitor of tyrosinase when L-tyrosine was used as a substrate. Its biosynthesis precursors and analogs, including glycitein, daidzein, and genistein, showed little anti-tyrosinase activity. The results suggest that hydroxyl groups at the C-6 and C-7 positions of the isoflavone skeleton might play an important role in the expression of tyrosinase inhibitory activity.

- Choi H, Ahn S, Lee BG, Chang I, Hwang JS.
Inhibition of skin pigmentation by an extract of Lepidium apetalum and its possible implication in IL-6 mediated signaling. Pigment Cell Res. 18(6):439-46, 2005.

The development of effective skin-lightening agents is an increasingly important area of research aimed at the treatment of hyperpigmentation induced by UV irradiation or by medical conditions such as melasma, postinflammatory melanoderma and solar lentigo. Although some inhibit tyrosinase, identifying and understanding the mechanisms of action of other agents is an important goal if more effective pigmentation inhibitors are to be developed. We present here that an extract of Lepidium apetalum (ELA) decreased UV-induced skin pigmentation in brown guinea pigs and melanogenesis of HM3KO human melanoma cells. Interestingly, ELA did not reduce melanogenesis in HM3KO cells unless they were co-cultivated in keratinocyte-conditioned medium prepared by culturing keratinocytes with ELA. Under these conditions, ELA decreased tyrosinase mRNA and protein expression as well as melanin content via an ELA-mediated increase in keratinocyte IL-6 production which in turn was shown to decrease in the expression Mitf, a transcription factor implicated in tyrosinase gene expression and melanocyte differentiation. The results reveal that ELA may be an effective inhibitor of hyperpigmentation caused by UV irradiation or by pigmented skin disorders through a mechanism involving IL-6-mediated downregulation of Mitf rather than a direct inhibition of tyrosinase activity.

- Finn GJ, Creaven BS, Egan DA.
Activation of mitogen activated protein kinase pathways and melanogenesis by novel nitro-derivatives of 7-hydroxycomarin in human malignant melanoma cells. Eur J Pharm Sci. 26(1):16-25, 2005.
6-Nitro-7-hydroxycoumarin (6-NO2-7-OHC) and 3,6,8-trinitro-7-hydroxycoumarin (3,6,8-NO2-7-OHC) have previously been shown to be potent and selective anti-proliferative agents in a human melanoma cell line. These agents functioned by decreasing DNA synthesis, through an inhibition of the S phase regulatory protein, cyclin A. However, the key molecular target(s) for these drugs remained undefined. Here, we attempted to elucidate the exact nature of the relationship between drug exposure and signal transduction, particularly their effects on the mitogen activated protein kinase (MAPK) cascades, and the consequent effect on cell growth, death and differentiation. Comparative studies were carried out using 7-hydroxycoumarin (7-OHC). Both nitro-derivatives were found to alter the phosphorylation status of ERK1/ERK2 and p38. However, 7-OHC exerted this effect only at higher concentrations and longer incubation times. Also, none of the three drugs had any effect on SAPK phosphorylation. Tyrosinase activity assays and morphological studies were used to show drug-induced effects on cellular differentiation. Unlike 7-OHC, both 6-NO2-7-OHC and 3,6,8-NO2-7-OHC caused a dramatic increase in tyrosinase activity in a manner similar to the cAMP elevating agent, forskolin. Also, the MEK inhibitor (PD98059) in combination with nitro-derivatives stimulated an even greater increase in tyrosinase activity when compared to either drug. In addition, the p38 inhibitor (SB203580) reduced the activity of both drugs. Morphological examination of treated cells showed nitro-derivatives caused changes consistent with altered cellular differentiation. Taken together, we have established that exposure of human malignant melanoma cells to these drugs leads to a modulation of p38 MAP kinase phosphorylation. This implies that these drugs may function by altering both melanogenesis and cellular differentiation. However, their effect on the levels of these proteins rather than their phosphorylation status remains to be determined. Therefore, additional studies are underway in order to identify the exact binding partners for these drugs.

- Forshew T, Khaliq S, Tee L, Smith U, Johnson CA, Mehdi SQ, Maher ER.
Identification of novel TYR and TYRP1 mutations in oculocutaneous albinism. Clin Genet. 68(2):182-4, 2005.

- Fu B, Li H, Wang X, Lee FS, Cui S.
Isolation and identification of flavonoids in licorice and a study of their inhibitory effects on tyrosinase. J Agric Food Chem. 53(19):7408-14, 2005.
Five different flavonoids were isolated from licorice after multistep chromatographic fractionation. The aim was to identify and characterize active components in licorice responsible for

antibrowning activities and to seek new tyrosinase inhibitors for applications as antibrowning and depigmenting agents in the food and cosmetic industries. The isolated flavonoids were identified as liquiritin, licuraside, isoliquiritin, liquiritigenin (from Glycyrrhiza uralensis Fisch.), and licochalcone A (from Glycyrrhiza inflate Bat.) by UV, MS, (1)H NMR, and (13)C NMR analyses. The inhibitory potencies and capacities of these flavonoids toward monophenolase activity of mushroom tyrosinase were investigated. The IC(50) values of licuraside, isoliquiritin, and licochalcone A for monophenolase activity were 0.072, 0.038, and 0.0258 mM, respectively. A study of the mechanisms of monophenolase inhibition by these flavonoids indicated that they are all competitive inhibitors. Different from the above flavonoids, no inhibitory activity was observed for liquiritin, whereas liquiritigenin activated the monophenolase activity as a cofactor. The inhibitory effect of licuraside, isoliquiritin, and licochalcone A on diphenolase activity with l-DOPA as the substrate was much lower than those with l-tyrosine. Results suggest that licuraside, isoliquiritin, and licochalcone A have the high potential to be further developed into effective antibrowning and depigmenting agents.

- Gandia-Herrero F, Escribano J, Garcia-Carmona F.
Characterization of the activity of tyrosinase on betaxanthins derived from (R)-amino acids. J Agric Food Chem. 53(23):9207-12, 2005.
The activity of tyrosinase (EC 1.14.18.1) on selected (R)-betaxanthins is characterized in depth, demonstrating that the activity of the enzyme is not restricted to betaxanthins derived from (S)-amino acids. Conversion of (R)-tyrosine-betaxanthin [(R)-portulacaxanthin II] to the pigment (R)-dopaxanthin and its further oxidation to a series of products is described. Compound identity was studied by high performance liquid chromatography and electrospray ionization-mass spectrometry. The reaction rate on the (R)-isomer of dopaxanthin is 1.9-fold lower than that obtained for the (S)-isomer in previous studies. Tyrosinase showed stereospecificity in its affinity toward betaxanthins. The characterization of the activity of tyrosinase on (R)-betaxanthins reinforces the role of the enzyme in the biosynthetic scheme of betalains.

- Gandia-Herrero F, Jimenez-Atienzar M, Cabanes J, Garcia-Carmona F, Escribano J.
Differential activation of a latent polyphenol oxidase mediated by sodium dodecyl sulfate. J Agric Food Chem. 53(17):6825-30, 2005.
A kinetic study of the activity of soluble and membrane-bound latent polyphenol oxidase (PPO) extracted from beet root (Beta vulgaris) was carried out. For the first time, two types of behavior (hyperbolic and sigmoid) are reported in the same enzyme for PPO activation by the surfactant sodium dodecyl sulfate (SDS), depending on substrate nature. A kinetic model based on cooperative systems is developed to describe the activation effect of SDS, enabling the determination of the number of surfactant molecules binding to the enzyme in the activation process. The results indicate that the active site of the enzyme is not affected by SDS and that a stepwise conformational change favors the access of hydrophobic substrates compared to hydrophilic ones. Differential activation of PPO mediated by SDS may be of relevance in the control of PPO activity since the enzyme is able to express activity toward a specific substrate while remaining latent to others.

- Garcia-Molina F, Penalver MJ, Rodriguez-Lopez JN, Garcia-Canovas F, Tudela J.
Enzymatic method with polyphenol oxidase for the determination of cysteine and N-acetylcysteine. J Agric Food Chem. 53(16):6183-9, 2005.
Thiols, such as cysteine and N-acetylcysteine, are included in many pharmaceutical products for their mucolytic properties. The method described here uses mushroom polyphenol oxidase (PPO) to determine two thiols and consists of measuring the lag period in the formation of the product generated as PPO acts on o-diphenol in the presence of a thiol. In the experimental conditions, o-quinone is formed enzymatically and then reacts stoichiometrically with the thiol, originating the corresponding thiol-diphenol adduct, which does not absorb visible light. Once the thiol has been used up, the o-quinone can be observed in the medium. It must be borne in mind that the inhibition

of PPO is practically null at low concentrations of thiol, and the only effect observed is the formation of the thiol-diphenol adduct. In the following, an exact kinetic method capable of rapidly and accurately assaying thiols with PPO and o-diphenol is optimized and is shown to be a straightforward way of calculating thiol concentration. The method has been successfully applied to the determination of cysteine in model solutions and of N-acetylcysteine in pharmaceutical products.

- Gheibi N, Saboury AA, Mansuri-Torshizi H, Haghbeen K, Moosavi-Movahedi AA.
  The inhibition effect of some n-alkyl dithiocarbamates on mushroom tyrosinase. J Enzyme Inhib Med Chem. 20(4):393-9, 2005.
  Three new n-alkyl dithiocarbamate compounds, as sodium salts, C4H9NHCS2Na (I), C6H13NHCS2Na (II) and C8H17NHCS2Na (III), were synthesized and examined for inhibition of both cresolase and catecholase activities of mushroom tyrosinase (MT) from a commercial source of Agaricus bisporus in 10 mM phosphate buffer pH 6.8, at 293K using UV spectrophotometry. Caffeic acid and p-coumaric acid were used as natural substrates for the enzyme for the catecholase and cresolase reactions, respectively. Lineweaver-Burk plots showed different patterns of mixed and competitive inhibition for catecholase and cresolase reactions, respectively. These new synthetic compounds can be classified as potent inhibitors of MT due to Ki values of 0.8, 1.0 and 1.8 microM for cresolase inhibitory activity, and also 9.4, 14.5 and 28.1 microM for catecholase inhibitory activity for I, II and III, respectively. They showed a greater potency in the inhibitory effect towards the cresolase activity of MT. Both substrate and inhibitor can be bound to the enzyme with negative cooperativity between the binding sites (alpha > 1) and this negative cooperativity increases with increasing length of the aliphatic tail in these compounds. The inhibition mechanism is presumably related to the chelating of the binuclear coppers at the active site and the different Ki values may be related to different interaction of the aliphatic chains of I, II and III with the hydrophobic pocket in the active site of the enzyme.

- Hernandez-Romero D, Solano F, Sanchez-Amat A.
  Polyphenol Oxidase Activity Expression in Ralstonia solanacearum. Appl Environ Microbiol. 71(11):6808-15, 2005.
  Sequencing of the genome of Ralstonia solanacearum revealed several genes that putatively code for polyphenol oxidases (PPOs). To study the actual expresión of these genes, we looked for and detected all kinds of PPO activities, including laccase, cresolase, and catechol oxidase activities, in cellular extracts of this microorganism. The conditions for the PPO assays were optimizad for the phenolic substrate, pH, and sodium dodecyl sulfate concentration used. It was demonstrated that three different PPOs are expressed. The genes coding for the enzymes were unambiguously correlated with the enzymatic activities detected by generation of null mutations in the genes by using insertional mutagenesis with a suicide plasmid and estimating the changes in the levels of enzymatic activities compared to the levels in the wild-type strain. The protein encoded by the RSp1530 locus is a multicopper protein with laccase activity. Two other genes, RSc0337 and RSc1501, code for nonblue copper proteins exhibiting homology to tyrosinases. The product of RSc0337 has strong tyrosine hydroxylase activity, and it has been shown that this enzyme is involved in melanin synthesis by R. solanacearum. The product of the RSc1501 gene is an enzyme that shows a clear preference for oxidation of o-diphenols. Preliminary characterization of the mutants obtained indicated that PPOs expressed by R. solanacearum may participate in resistance to phenolic compounds since the mutants exhibited higher sensitivity to l-tyrosine than the wild-type strain. These results suggest a possible role in the pathogenic process to avoid plant resistance mechanisms involving the participation of phenolic compounds.

- Khan KM, Maharvi GM, Khan MT, Jabbar Shaikh A, Perveen S, Begum S, Choudhary MI.
  Tetraketones: A new class of tyrosinase inhibitors. Bioorg Med Chem. 2005 Sep 27; [Epub ahead of print]

Twenty-eight tetraketones (1-28) with variable substituents at C-7 were synthesized and evaluated as tyrosinase inhibitors. Remarkably compounds 25 (IC(50)=2.06muM), 11 (IC(50)=2.09muM), 15 (IC(50)=2.61muM), and 27 (IC(50)=3.19muM) were found to be the most active compounds of the series, even better than both standards kojic acid (IC(50)=16.67muM) and l-mimosine (IC(50)=3.68muM). This study may lead to the discovery of therapeutically potent agents against clinically very important dermatological disorders including hyperpigmentation as well as skin melanoma.

- Kim DS, Park SH, Kwon SB, Youn SW, Park ES, Park KC.
Heat treatment decreases melanin synthesis via protein phosphatase 2 inactivation. Cell Signal. 17(8):1023-31, 2005. Epub 2004 Dec 23.
In the present study, we investigated the effects of heat treatment on melanogenesis in a mouse melanocyte cell line (Mel-Ab). It has been reported that activated extracellular signal-regulated kinase (ERK) is responsible for microphthalmia-associated transcription factor (MITF) degradation, which leads to a reduction in tyrosinase protein production and melanin synthesis. Here we demonstrate that heat treatment induces sustained ERK activation, which may inhibit melanogenesis. However, the specific ERK pathway inhibitors, PD98059 or U0126 did not restore heat-induced hypopigmentation. Furthermore, PD98059 or U0126 hardly blocked the heat-induced activation of ERK. These results suggest that heat treatment may inactivate protein phosphatase, and thus ERK activation is maintained. To support this hypothesis, we examined the effects of heat treatment on protein phosphatase 2A (PP2A) activity. The results obtained show that heat treatment inactivates PP2A, which may subsequently cause ERK activation and that heat treatment inhibits MITF promoter activity. Overall, our results demonstrate that heat treatment reduces melanin production in a temperature-dependent manner.

- Kim HJ, Seo SH, Lee BG, Lee YS.
Identification of tyrosinase inhibitors from Glycyrrhiza uralensis. Planta Med. 71(8):785-7, 2005.
Tyrosinase is a key enzyme in the production of melanins. Phytochemical studies of a Glycyrrhiza uralensis extract were performed by measuring the tyrosinase and melanin synthesis inhibitory activity. Glycyrrhisoflavone and glyasperin C were identified as tyrosinase inhibitors for the first time. Glyasperin C showed a stronger tyrosinase inhibitory activity (IC (50) = 0.13 +/- 0.01 microg/mL) than glabridin (IC (50) = 0.25 +/- 0.01 microg/mL) and a moderate inhibition of melanin production (17.65 +/- 8.8 % at 5 microg/mL). Glycyrrhisoflavone showed a strong melanin synthesis inhibitory activity (63.73 +/- 6.8 % inhibition at 5 microg/mL). These results suggest that glyasperin C and glycyrrhisoflavone could be promising candidates in the design of skin-whitening agents.

- Kim YJ, Uyama H.
Tyrosinase inhibitors from natural and synthetic sources: structure, inhibition mechanism and perspective for the future. Cell Mol Life Sci. 62(15):1707-23, 2005.
Tyrosinase is known to be a key enzyme in melanin biosynthesis, involved in determining the color of mammalian skin and hair. Various dermatological disorders, such as melasma, age spots and sites of actinic damage, arise from the accumulation of an excessive level of epidermal pigmentation. In addition, unfavorable enzymatic browning of plant-derived foods by tyrosinase causes a decrease in nutritional quality and economic loss of food products. The inadequacy of current conventional techniques to prevent tyrosinase action encourages us to seek new potent tyrosinase inhibitors. This article overviews the various inhibitors obtained from natural and synthetic sources with their industrial importance.

- Komarov DA, Slepneva IA, Glupov VV, Khramtsov VV.
Superoxide and hydrogen peroxide formation during enzymatic oxidation of DOPA by phenoloxidase. Free Radic Res. 39(8):853-8, 2005.

Generation of superoxide anion and hydrogen peroxide during enzymatic oxidation of 3-(3,4-dihydroxyphenyl)-DL-alanine (DOPA) has been studied. The ability of DOPA to react with O2*- has been revealed. EPR spectrum of DOPA-semiquinone formed upon oxidation of DOPA by O2*- was observed using spin stabilization technique of ortho-semiquinones by Zn2+ ions. Simultaneously, the oxidation of DOPA by O2*- was found to produce hydrogen peroxide (H2O2). The analysis of H2O2 formation upon oxidation of DOPA by O2*- using 1-hydroxy-3-carboxy-pyrrolidine (CP-H), and SOD as competitive reagents for superoxide provides consistent values of the rate constant for the reaction between DOPA and O2*- being equal to (3.4+/-0.6)x10(5) M(-1) s(-1).The formation of H2O2 during enzymatic oxidation of DOPA by phenoloxidase (PO) has been shown. The H2O2 production was found to be SOD-sensitive. The inhibition of H2O2 production by SOD was about 25% indicating that H2O2 is produced both from superoxide anion and via two-electron reduction of oxygen at the enzyme. The attempts to detect superoxide production during enzymatic oxidation of DOPA using a number of spin traps failed apparently due to high value of the rate constant for DOPA interaction with O2*-.

- Koval IA, Belle C, Selmeczi K, Philouze C, Saint-Aman E, Schuitema AM, Gamez P, Pierre J, Reedijk J.
  Catecholase activity of a mu-hydroxodicopper(II) macrocyclic complex: structures, intermediates and reaction mechanism. J Biol Inorg Chem. 10(7):739-50, 2005.
  The monohydroxo-bridged dicopper(II) complex (1), its reduced dicopper(I) analogue (2) and the trans-mu-1,2-peroxo-dicopper(II) adduct (3) with the macrocyclic N-donor ligand [22]py4pz (9,22-bis(pyridin-2'-ylmethyl)-1,4,9,14,17,22,27,28,29,30- decaazapentacyclo -[22.2.1(14,7).1(11,14) .1(17,20)] triacontane-5,7(28),11(29),12,18,20(30), 24(27),25-octaene), have been prepared and characterized, including a 3D structure of 1 and 2. These compounds represent models of the three states of the catechol oxidase active site: met, deoxy (reduced) and oxy. The dicopper(II) complex 1 catalyzes the oxidation of catechol model substrates in aerobic conditions, while in the absence of dioxygen a stoichiometric oxidation takes place, leading to the formation of quinone and the respective dicopper(I) complex. The catalytic reaction follows a Michaelis-Menten behavior. The dicopper(I) complex binds molecular dioxygen at low temperature, forming a trans-mu-1,2-peroxo-dicopper adduct, which was characterized by UV-Vis and resonance Raman spectroscopy and electrochemically. This peroxo complex stoichiometrically oxidizes a second molecule of catechol in the absence of dioxygen. A catalytic mechanism of catechol oxidation by 1 has been proposed, and its relevance to the mechanisms earlier proposed for the natural enzyme and other copper complexes is discussed.

- Lavado A, Montoliu L.
  New animal models to study the role of tyrosinase in normal retinal development. Front Biosci. 11:743-52, 2006.
  Albino animals display a hypopigmented phenotype associated with several visual abnormalities, including rod photoreceptor cell deficits, abnormal patterns of connections between the eye and the brain and a general underdevelopment of central retina. Oculocutaneous albinism type I, a common form of albinism, is caused by mutations in the tyrosinase gene. In mice, the albino phenotype can be corrected by functional tyrosinase transgenes. Tyrosinase transgenic animals not only show normal pigmentation but the correction of all visual abnormalities associated with albinism, confirming a role of tyrosinase, a key enzyme in melanin biosynthesis, in normal retinal development. Here, we will discuss recent work carried out with new tyrosinase transgenic mouse models, to further analyse the role of tyrosinase in retinal development. We will first report a transgenic model with inducible tyrosinase expression that has been used to address the regulated activation of this gene and its associated effects on the development of the visual system. Second, we will comment on an interesting yeast artificial chromosome (YAC)-tyrosinase transgene, lacking important regulatory elements, that has highlighted the significance of local interactions between the retinal pigment epithelium (RPE) and developing neural retina.

- Lin WP, Lai HL, Liu YL, Chiung YM, Shiau CY, Han JM, Yang CM, Liu YT.
Effect of melanin produced by a recombinant Escherichia coli on antibacterial activity of antibiotics. J Microbiol Immunol Infect. 38(5):320-6, 2005.
A recombinant plasmid, pYL-1, containing a tyrosinase gene whose expression is under the control of a phage T5 promoter and 2 lac operators, was constructed. Escherichia coli JM109 harboring pYL-1 was used for production of bacterial melanin. A simple procedure for the isolation and purification of melanin was developed. The ultraviolet (UV)-visible light absorption spectra of melanin prepared by chemical synthesis and derived from different organisms, including bacteria, a plant and an animal source, were determined. Melanins produced by both bacteria and chemical synthesis showed a steady increase of absorption at wavelengths of UV light ranging from approximately 200-400 nm, while melanin derived either from plant or animal sources showed an additional discrete absorption peak at wavelength 280 nm upon a similar steady increase of absorption. This additional absorption peak could be due to the presence of protein-bound melanins in animal and plant sources while a free form of melanin was obtained from bacteria and chemical synthesis. Analysis of the effect of bacterial melanin on the activity of antibiotics against E. coli revealed that the activities of polymyxin B, kanamycin, tetracycline, and ampicillin were markedly reduced in the presence of melanin, whereas the activity of norfloxacin was not affected. The reduction of the antibacterial activity may result directly from the interaction of antibiotics with melanin. However, the mechanism of this interaction remains to be demonstrated.

- Lyons LA, Foe IT, Rah HC, Grahn RA.
Chocolate coated cats: TYRP1 mutations for brown color in domestic cats. Mamm Genome. 16(5):356-66, 2005.
Brown coat color phenotypes caused by mutations in tyrosinase-related protein-1 (TYRP1) are recognized in many mammals. Brown variations are also recognized in the domestic cat, but the causative mutations are unknown. In cats, Brown, B, has a suggested allelic series, B > b > b1. The B allele is normal wild-type black coloration. Cats with the brown variation genotypes, bb or bb1, are supposedly phenotypically chocolate (aka chestnut) and the light brown genotype, b1b1, are supposedly phenotypically cinnamon (aka red). The complete coding sequence of feline TYRP1 and a portion of the 5' UTR was analyzed by direct sequencing of genomic DNA of wild-type and brown color variant cats. Sixteen single nucleotide polymorphisms (SNPs) were identified. Eight SNPs were in the coding regions, six are silent mutations. Two exon 2 on mutations cause amino acid changes. The C to T nonsense mutation at position 298 causes an arginine at amino acid 100 to be replaced by the opal (UGA) stop codon. This mutation is consistent with the cinnamon phenotype and is the putative light brown, b1, mutation. An intron 6 mutation that potentially disrupts the exon 6 downstream splice-donor recognition site is associated with the chocolate phenotype and is the putative brown, b, mutation. The allelic series was confirmed by segregation and sequence analyses. Three microsatellite makers had significant linkage to the brown phenotype and two for the TYRP1 mutations in a 60-member pedigree. These mutations could be used to identify carriers of brown phenotypes in the domestic cat.

- Maresca V, Flori E, Cardinali G, Briganti S, Lombardi D, Mileo AM, Paggi MG, Picardo M.
Ferritin light chain down-modulation generates depigmentation in human metastatic melanoma cells by influencing tyrosinase maturation. J Cell Physiol. 2005 Oct 26; [Epub ahead of print]
Recently, after the identification of ferritin light chain (L-ferritin) gene and protein over-expression in human metastatic melanoma cells, we engineered, starting from the LM metastatic melanoma cell line, clones in which L-ferritin gene expression was down-regulated by the stable expression of a specific antisense construct. The present investigation started from the observation that L-ferritin down-regulated LM cells displayed a less pigmented phenotype, confirmed by a major decrease of total melanin, when compared to control LM cells. This finding was accompanied by a dramatic decrease in tyrosinase activity, which was not paralleled by a concomitant reduction of the amount of tyrosinase specific mRNA. Western blot analysis of tyrosinase in control LM cells

displayed a pattern, which corresponds to the progressive glycosylation of the native protein up to the 80 kDa form, considered the functional one. Tyrosinase pattern assayed in L-ferritin down-regulated LM cells showed the remarkable absence of the 80 kDa form and a prevalence of endoglycosidase H (endo H)-sensitive immature (70 kDa) tyrosinase, accumulated in the endoplasmic reticulum (ER), as confirmed by confocal microscopy analysis. These results demonstrate that, in a human metastatic melanoma cell line, the stress condition promoted by L-ferritin down-modulation, can substantially influence proper maturation of tyrosinase.

- Mastore M, Kohler L, Nappi AJ.
  Production and utilization of hydrogen peroxide associated with melanogenesis and tyrosinase-mediated oxidations of DOPA and dopamine. FEBS J. 272(10):2407-15, 2005.
  The synthesis and involvement of H(2)O(2) during the early stages of melanogenesis involving the oxidations of DOPA and dopamine (diphenolase activity) were established by two sensitive and specific electrochemical detection systems. Catalase-treated reaction mixtures showed diminished rates of H(2)O(2) production during the autoxidation and tyrosinase-mediated oxidation of both diphenols. Inhibition studies with the radical scavenger resveratrol revealed the involvement in these reactions of additional reactive intermediate of oxygen (ROI), one of which appears to be superoxide anion. There was no evidence to suggest that H(2)O(2) or any other ROI was produced during the tyrosinase-mediated conversion of tyrosine to DOPA (monophenolase activity). Establishing by electrochemical methods the endogenous production H(2)O(2) in real time confirms recent reports, based in large part on the use of exogenous H(2)O(2), that tyrosinase can manifest both catalase and peroxidase activities. The detection of ROI in tyrosinase-mediated in vitro reactions provides evidence for sequential univalent reductions of O(2), most likely occurring at the enzyme active site copper. Collectively, these observations focus attention on the possible involvement of peroxidase-H(2)O(2) systems and related ROI-mediated reactions in promoting melanocytotoxic and melanoprotective processes.

- Mirica LM, Vance M, Rudd DJ, Hedman B, Hodgson KO, Solomon EI, Stack TD.
  Tyrosinase reactivity in a model complex: an alternative hydroxylation mechanism. Science. 308(5730):1890-2, 2005.
  The binuclear copper enzyme tyrosinase activates O2 to form a mu-eta2:eta2-peroxodicopper(II) complex, which oxidizes phenols to catechols. Here, a synthetic mu-eta2:eta2-peroxodicopper(II) complex, with an absorption spectrum similar to that of the enzymatic active oxidant, is reported to rapidly hydroxylate phenolates at -80 degrees C. Upon phenolate addition at extreme temperature in solution (-120 degrees C), a reactive intermediate consistent with a bis-mu-oxodicopper(III)-phenolate complex, with the O-O bond fully cleaved, is observed experimentally. The subsequent hydroxylation step has the hallmarks of an electrophilic aromatic substitution mechanism, similar to tyrosinase. Overall, the evidence for sequential O-O bond cleavage and C-O bond formation in this synthetic complex suggests an alternative intimate mechanism to the concerted or late stage O-O bond scission generally accepted for the phenol hydroxylation reaction performed by tyrosinase.

- Miyamura Y, Verma IC, Saxena R, Hoshi M, Murase A, Nakamura E, Kono M, Suzuki T, Yasue S, Shibata S, Sakakibara A, Tomita Y.
  Five novel mutations in tyrosinase gene of Japanese and Indian patients with oculocutaneous albinism type I (OCA1). J Invest Dermatol. 125(2):397-8, 2005.

- Ni-Komatsu L, Leung JK, Williams D, Min J, Khersonsky SM, Chang YT, Orlow SJ.
  Triazine-based tyrosinase inhibitors identified by chemical genetic screening. Pigment Cell Res. 18(6):447-53, 2005.
  As most of the available depigmenting agents exhibit only modest activity and some exhibit toxicities that lead to adverse side effects after long-term usage, there remains a need for novel depigmenting agents. Chemical genetic screening was performed on cultured melanocytes to identify novel depigmenting compounds. By screening a tagged-triazine library, we identified four

compounds, TGH11, TGD10, TGD39 and TGJ29, as potent pigmentation inhibitors with IC(50) values in the range of 10 muM. These newly identified depigmenting compounds were found to function as reversible inhibitors of tyrosinase, the key enzyme involved in melanin synthesis. Tyrosinase was further confirmed as the cellular target of these compounds by affinity chromatography. Kinetic data suggest that all four compounds act as competitive inhibitors of tyrosinase, most likely competing with l-3,4-dihydroxyphenylalanine (l-DOPA) for binding to the DOPA-binding site of the enzyme. No effect on levels of tyrosinase protein, processing or trafficking was observed upon treatment of melanocytes with these compounds. Cytotoxicity was not observed with these compounds at concentrations up to 20 muM. Our data suggest that TGH11, TGD10, TGD39 and TGJ29 are novel potent tyrosinase inhibitors with potential beneficial effects in the treatment of cutaneous hyperpigmentation.

- Ni-Komatsu L, Orlow SJ.
Heterologous expression of tyrosinase recapitulates the misprocessing and mistrafficking in oculocutaneous albinism type 2: Effects of altering intracellular pH and pink-eyed dilution gene expression. Exp Eye Res. 2005 Sep 29; [Epub ahead of print]
The processing and trafficking of tyrosinase, a melanosomal protein essential for pigmentation, was investigated in a human epithelial 293 cell line that stably expresses the protein. The effects of the pink-eyed dilution (p) gene product, in which mutations result in oculocutaneous albinism type 2 (OCA2), on the processing and trafficking of tyrosinase in this cell line were studied. The majority of tyrosinase was retained in the endoplasmic reticulum-Golgi intermediate compartment and the early Golgi compartment in the 293 cells expressing the protein. Coexpression of p could partially correct the mistrafficking of tyrosinase in 293 cells. Tyrosinase was targeted to the late endosomal and lysosomal compartments after treatment of the cells with compounds that correct the tyrosinase mistrafficking in albino melanocytes, most likely through altering intracellular pH, while the substrate tyrosine had no effect on the processing of tyrosinase. Remarkably, this heterologous expression system recapitulates the defective processing and mistrafficking of tyrosinase observed in OCA2 albino melanocytes and certain amelanotic melanoma cells. Coexpression of other melanosomal proteins in this heterologous system may further aid our understanding of the details of normal and pathologic processing of melanosomal proteins.

- Obata H, Ishida H, Hata Y, Kawato A, Abe Y, Akao T, Akita O, Ichishima E.
Cloning of a novel tyrosinase-encoding gene (melB) from Aspergillus oryzae and its overexpression in solid-state culture (Rice Koji). J Biosci Bioeng. 97(6):400-5, 2004.
We have cloned a novel tyrosinase-encoding gene (melB) specifically expressed in solid-state culture of Aspergillus oryzae. A tyrosinase-encoding gene (melO) from A. oryzae was already cloned and the protein structures of its catalytic and copper binding domains were investigated. However, our recent results revealed that the melO gene was highly expressed in submerged culture but not in solid-state culture. Because tyrosinase activity was also detected in solid-state culture, we assumed that another tyrosinase gene other than melO is expressed in solid-state culture. Another tyrosinase gene was screened using the expressed sequence tag (EST) library. One redundant cDNA clone homologous with the tyrosinase gene was found in the collection of wheat bran culture. Northern blot analysis revealed that the gene corresponding to the cDNA clone was specifically expressed in solid-state culture (koji making), but not in submerged culture. Molecular cloning showed that the gene carried six exons interrupted by five introns and had an open reading frame encoding 616 amino acid residues. This gene was designated as melB. The deduced amino acid sequence of the gene had weak homology (24%-33%) with MelO and other fungal tyrosinases but the sequences of the copper binding domains were highly conserved. When the melB gene was expressed under the control of the glaB promoter in solid-state culture, tyrosinase activity was markedly enhanced and the culture mass was browned with the melanization by MelB tyrosinase. These results indicated that the melB gene encodes a novel tyrosinase associated with melanization in solid-state culture.

- Ohguchi K, Banno Y, Nakagawa Y, Akao Y, Nozawa Y.
  Negative regulation of melanogenesis by phospholipase D1 through mTOR/p70 S6 kinase 1 signaling in mouse B16 melanoma cells. J Cell Physiol. 205(3):444-51, 2005.
  Melanogenesis is a principal parameter of differentiation in melanocytes and melanoma cells. Our recent study has demonstrated that phospholipase D1 (PLD1) regulates the melanogenic signaling through modulating the expression of tyrosinase, the rate-limiting step enzyme in the melanin biosynthesis. The current study was designed to gain more insight into the involvement of PLD1 in the regulation of melanogenesis. To investigate the role of PLD1, we examined the effect of knockdown of endogenous PLD1 by small interference RNA (siRNA) on melanogenesis in B16 melanoma cells. It was shown that the melanin synthesis was induced in PLD1-knockdowned cells, and also that the level of melanin synthesis was well correlated with increases in expression level of tyrosinase and its related proteins (Tyrp1 and Dct). Furthermore, the reduction of expresión levels of PLD1 by siRNA transfection was accompanied by diminution of ribosomal S6 kinase 1 (S6K1) phosphorylation. The activity of mammalian target of rapamycin (mTOR) is essential for phosphorylation of S6K1 and the treatment malanoma cells with rapamycin, a potent inhibitor of mTOR effectively induced melanogenesis. The results obtained here provide possible evidence that PLD1 exerts a negative regulatory role in the melanogenic process through mTOR/S6K1 signaling.

- Park KH, Park YD, Lee JR, Hahn HS, Lee SJ, Bae CD, Yang JM, Kim DE, Hahn MJ.
  Inhibition kinetics of mushroom tyrosinase by copper-chelating ammonium tetrathiomolybdate. Biochim Biophys Acta. 1726(1):115-20, 2005. Epub 2005 Jul 5.
  With a strategy of chelating coppers at tyrosinase active site to detect an effective inhibitor, several copper-specific chelators were applied in this study. Ammonium tetrathiomolybdate (ATTM) among them, known as a drug for treating Wilson's disease, turned out to be a significant tyrosinase inhibitor. Treatment with ATTM on mushroom tyrosinase completely inactivated enzyme activity in a dose-dependent manner. Progress-of-substrate reaction kinetics using the two-step kinetic pathway and dilution of the ATTM revealed that ATTM is a tight-binding inhibitor and high dose of ATTM irreversibly inactivated tyrosinase. Progress-of-substrate reaction kinetics and activity restoration with a dilution of the ATTM indicated that the copper-chelating ATTM may bind slowly but reversibly to the active site without competition with substrate, and the enzyme-ATTM complex subsequently undergoes reversible conformational change, leading to complete inactivation of the tyrosinase activity. Thus, inhibition by ATTM on tyrosinase could be categorized as complexing type of inhibition with a slow and reversible binding. Detailed analysis of inhibition kinetics provided IC50 at the steady-state and inhibitor binding constant (K(I)) for ATTM as 1.0+/-0.2 microM and 10.65 microM, respectively. Our results may provide useful information regarding effective inhibitor of tyrosinase as whitening agents in the cosmetic industry.

- Pezzella A, Lista L, Napolitano A, d'Ischia M.
  Tyrosinase-catalyzed oxidation of 17beta-estradiol: structure elucidation of the products formed beyond catechol estrogen quinones. Chem Res Toxicol. 18(9):1413-9, 2005.
  This paper reports a systematic characterization of the products formed by oxidation of 17beta-estradiol (1) with tyrosinase/O2 at low concentrations of physiological relevance. With the substrate at 1-10 nM concentration, the main reaction products included, beside the catechol estrogens 2-hydroxyestradiol (2) and 4-hydroxyestradiol (3), 6-oxo-2-hydroxyestradiol (4), 9,11-dehydro-2-hydroxyestradiol (6), 6,7-dehydro-2-hydroxyestradiol (7), and 9,11-dehydro-4-hydroxyestradiol (10). At higher estradiol concentrations, e.g., 1 microM, 6,7,8,9-dehydro-2-hydroxyestradiol (5) and the dimeric products 8 and 9 were also formed. The origin of these products from oxidative routes of 2 and 3 was established. Overall, the results of this study disclose novel aspects of the reactivity of 1 with the tyrosinase/O2 system and provide the first inventory of the oxidation products of catechol estrogen quinones.

- Smith JW, Koshoffer A, Morris RE, Boissy RE.
  Membranous complexes characteristic of melanocytes derived from patients with Hermansky-Pudlak syndrome type 1 are macroautophagosomal entities of the lysosomal compartment. Pigment Cell Res. 18(6):417-26, 2005.
  Hermansky-Pudlak syndrome (HPS) is an autosomal recessive disorder resulting from mutations in a family of genes required for efficient transport of lysosomal-related proteins from the trans-Golgi network to a target organelle. To date, there are several genetically distinct forms of HPS. Many forms of HPS exhibit aberrant trafficking of melanosome-targeted proteins resulting in incomplete melanosome biogenesis responsible for oculocutaneous albinism observed in patients. In HPS-1, melanosome-targeted proteins are localized to characteristic membranous complexes, which have morphologic similarities to macroautophagosomes. In this report, we evaluated the hipótesis that HPS-1-specific membranous complexes comprise a component of the lysosomal compartment of melanocytes. Using indirect immunofluorescence, an increase in co-localization of misrouted tyrosinase with cathepsin-L, a lysosomal cysteine protease, occurred in HPS-1 melanocytes. In addition, ribophorin II, an integral endoplasmic reticulum protein that is also a component of macroautophagosomes, and LC3, a specific marker of macrophagosomes, demonstrated localization to membranous complexes in HPS-1 melanocytes. At the electron microscopic level, the membranous complexes exhibited acid phosphatase activity and localization of exogenously supplied horseradish peroxidase (HRP)-conjugated gold particles, indicating incorporation of lysosomal and endosomal components to membranous complexes, respectively. These results confirm that membranous complexes of HPS-1 melanocytes are macroautophagosomal representatives of the lysosomal compartment.

- Spencer JD, Chavan B, Marles LK, Kauser S, Rokos H, Schallreuter KU.
  A novel mechanism in control of human pigmentation by β-melanocyte-stimulating hormone and 7-tetrahydrobiopterin. J Endocrinol.187(2):293-302, 2005.
  The human skin holds the full machinery for pro-opiomelanocortin processing. The alpha-melanocyte-stimulating hormone (alpha-MSH)/melanocortin-1-receptor cascade has been implicated as a major player via the cAMP signal in the control of melanogenesis. Only very recently the beta-endorphin/mu-opiate receptor signal has been added to the list of regulators of melanocyte dendricity and melanin formation. In this context it was reported that (6R)-l-erythro-5,6,7,8-tetrahydrobiopterin (6BH(4)) can act as an allosteric inhibitor of tyrosinase, the key enzyme in melanogenesis, and this inhibition is reversible by both alpha- and beta-MSH. It was also shown earlier that 7BH(4), the isomer of 6BH(4), is twice as active in this inhibition reaction. However, as yet it is not known whether 7BH(4) is indeed present in loco in the melanosome. We here provide evidence that this isomer is present in this organelle in a concentration range up to 50 x 10(-6) M. Determination of beta-MSH in melanosomal extracts yielded 10 pg/mg protein. Moreover, we demonstrate reactivation of the 7BH(4)/tyrosinase inhibitor complex by beta-MSH, whereas alpha-MSH failed to do so. Furthermore, we show intra-melanosomal l-dopa formation from dopachrome by 7BH(4) in a concentration range up to 134 x 10(-6) M. Based on these results, we propose a new receptor-independent mechanism in the control of tyrosinase/melanogenesis by beta-MSH and the pterin 7BH(4).

- Suzuki H, Furusho Y, Higashi T, Ohnishi Y, Horinouchi S.
  A novel ortho-aminophenol oxidase responsible for formation of phenoxazinone chromophore of grixazone. J Biol Chem. 2005 Nov 10; [Epub ahead of print]
  Grixazone, containing a phenoxazinone chromophore, is a secondary metabolite produced by Streptomyces griseus. In the grixazone biosynthesis gene cluster, griF encoding a tyrosinase homologue and griE encoding a protein similar to copper chaperons for tyrosinases are encoded. Expression study of GriE and GriF in Escherichia coli showed that GriE activated GriF by transferring copper ions to GriF, as is observed for a Streptomyces melanogenesis system in which MelC1 copper chaperon transfers Cu ions to MelC2 tyrosinase. In contrast with tyrosinases, GriF showed no monophenolase activity, although it oxidized various o-aminophenols as preferable

substrates rather than catechol-type substrates. Deletion of the griEF locus on the chromosome resulted in accumulation of 3-amino-4-hydroxybenzaldehyde (3,4-AHBAL) and its acetylated compound, 3-acetylamino-4-hydroxybenzaldehyde. GriF oxidized 3,4-AHBAL to yield an o-quinone imine derivative, which was then non-enzymatically coupled with another molecule of the o-quinone imine to form a phenoxazinone. Co-existence of N-acetylcysteine in the in vitro oxidation of 3,4-AHBAL by GriF resulted in the formation of grixazone A, which suggests that the SH group of N-acetylcysteine is conjugated to the o-quinone imine formed from 3,4-AHBAL, and the conjugate is presumably coupled with another molecule of the o-quinone imine. GriF is thus a novel o-aminophenol oxidase that is responsible for the formation of the phenoxazinone chromophore in the grixazone biosynthetic pathway.

- Theos AC, Tenza D, Martina JA, Hurbain I, Peden AA, Sviderskaya EV, Stewart A, Robinson MS, Bennett DC, Cutler DF, Bonifacino JS, Marks MS, Raposo G.
  Functions of Adaptor Protein (AP)-3 and AP-1 in Tyrosinase Sorting from Endosomes to Melanosomes. Mol Biol Cell. 16(11):5356-72, 2005. Epub 2005 Sep 14.
  Specialized cells exploit adaptor protein complexes for unique post-Golgi sorting events, providing a unique model system to specify adaptor function. Here, we show that AP-3 and AP-1 function independently in sorting of the melanocyte-specific protein tyrosinase from endosomes to the melanosome, a specialized lysosome-related organelle distinguishable from lysosomes. AP-3 and AP-1 localize in melanocytes primarily to clathrin-coated buds on tubular early endosomes near melanosomes. Both adaptors recognize the tyrosinase dileucine-based melanosome sorting signal, and tyrosinase largely colocalizes with each adaptor on endosomes. In AP-3-deficient melanocytes, tyrosinase accumulates inappropriately in vacuolar and multivesicular endosomes. Nevertheless, a substantial fraction still accumulates on melanosomes, concomitant with increased association with endosomal AP-1. Our data indicate that AP-3 and AP-1 function in partially redundant pathways to transfer tyrosinase from distinct endosomal subdomains to melanosomes and that the AP-3 pathway ensures that tyrosinase averts entrapment on internal membranes of forming multivesicular bodies.

- Theos AC, Truschel ST, Raposo G, Marks MS.
  The Silver locus product Pmel17/gp100/Silv/ME20: controversial in name and in function. Pigment Cell Res. 18(5):322-36, 2005.
  Mouse coat color mutants have led to the identification of more than 120 genes that encode proteins involved in all aspects of pigmentation, from the regulation of melanocyte development and differentiation to the transcriptional activation of pigment genes, from the enzymatic formation of pigment to the control of melanosome biogenesis and movement [Bennett and Lamoreux (2003) Pigment Cell Res. 16, 333]. One of the more perplexing of the identified Mouse pigment genes is encoded at the Silver locus, first identified by Dunn and Thigpen [(1930) J. Heredity 21, 495] as responsible for a recessive coat color dilution that worsened with age on black backgrounds. The product of the Silver gene has since been discovered numerous times in different contexts, including the initial search for the tyrosinase gene, the characterization of major melanosome constituents in various species, and the identification of tumor-associated antigens from melanoma patients. Each discoverer provided a distinct name: Pmel17, gp100, gp95, gp85, ME20, RPE1, SILV and MMP115 among others. Although all its functions are unlikely to have yet been fully described, the protein clearly plays a central role in the biogenesis of the early stages of the pigment organelle, the melanosome, in birds, and mammals. As such, we will refer to the protein in this review simply as pre-melanosomal protein (Pmel). This review will summarize the structural and functional aspects of Pmel and its role in melanosome biogenesis.

- Wang T, Waters CT, Jakins T, Yates JR, Trump D, Bradshaw K, Moore AT.
  Temperature sensitive oculocutaneous albinism associated with missense changes in the tyrosinase gene. Br J Ophthalmol. 89(10):1383-4, 2005.

- Zhao M, Soderhall I, Park JW, Ma YG, Osaki T, Ha NC, Wu CF, Soderhall K, Lee BL.
A novel 43-kDa protein as a negative regulatory component of phenoloxidase-induced melanin synthesis. J Biol Chem. 280(26):24744-51, 2005. Epub 2005 Apr 27.
The melanization reaction induced by activated phenoloxidase in arthropods is important in the multiple host defense innate immune reactions, leading to the sequestration and killing of invading microorganisms. This reaction ought to be tightly controlled because excessive formation of quinones and systemic hypermelanization are deleterious to the hosts, suggesting that a negative regulator(s) of melanin synthesis may exist in hemolymph. Here, we report the purification and cloning of a cDNA of a novel 43-kDa protein, from the meal-worm Tenebrio molitor, which functions as a melanization-inhibiting protein (MIP). The deduced amino acid sequence of 352 residues has no homology to known sequences in protein data bases. When the concentration of the 43-kDa protein was examined by Western blot analysis in a melanin-induced hemolymph prepared by injection of Candida albicans into T. molitor larvae, the 43-kDa protein specifically decreased in the melanin-induced hemolymph compared with control hemolymph. Recombinant MIP expressed in a baculovirus system had an inhibitory effect on melanin synthesis in vitro. RNA interference using a synthetic 445-mer double-stranded RNA of MIP injected into Tenebrio larvae showed that melanin synthesis was markedly induced. These results suggest that this 43-kDa MIP inhibits the formation of melanin and thus is a modulator of the melanization reaction to prevent the insect from excessive melanin synthesis in places where it should be inappropriate.

8. Melanosomes

(Prof. J. Borovansky)

Excellent review summarizing what is known about the protein product of the *OA1* gene displaying structural and functional features of G-protein-coupled receptor (GPCR), which is targetted not to plasma membrane but to lysosomes and melanosomes, has appeared. The OA1 represents the first example of exclusively intracellular GPCR (*Schiaffino et al.*). Signalling pathways that upregulate melanization in the retinal pigment epithelium and which may also be implicated in the downregulation of rod outer segments by the RPE were summarized by *Sarangajaran & Apte. Theos et al* reviewed what had been known about the structure and function of Pmel17/gp100/Silv/ME20 involved in melanosome biogenesis. The most important and unprecedented article dealing with melanosomal Pmel17 was published by *Fowler et al* who demonstrated that melanosomal fibres consisting of Mα fragment had amyloid structure. The discovery of abundant mammalian amyloid structure, that functions in melanosome biogenesis, challenges the current view that amyloid in mammalians is always cytotoxic. Three papers studied melanosome transfer (*Levi et al, Berens et al and Hachiya et al*). *Slc7a* gene was identified as a major regulator of phaeomelanin production (*Chintala et al.*). Sorting of tyrosinase from endosomes to melanosomes was studied by *Theos et al.* N-(2-diethylaminoethyl)-4-iodobenzamide extended the group of compounds with affinity to melanosomes (*Chéhadé et al.*). PC12 cells loaded with dopamine melanin were suggested as an in vitro model for studies on the role of neuromelanin for the toxicity of chemicals in pigmented neurons (*Östergren et al*.)

Melanosomes are a differentiation marker not only of melanoma, but they can be also present in other tumours such as schwannoma (*Zhang et al*), neurofibroma, Bednar tumour, meningeal melanocytoma, paraganglioma and tumours of the perivascular epithelial cells (PEComas). PEComas are characterized by immunohistochemical profile of negativity for epithelial markers and positivity for melanocyte markers – HMB-45 and Mart-1/Melan A and by the presence (*Iyengar et al)* or absence of (pre)melanosomes (*Fukunaga*).

- Berens W, Van Den Bossche K, Yoon TJ, Westbroek W, Valencia JC, Out CJ, Naeyaert JM, Hearing VJ, Lambert J.
  Different approaches for assaying melanosome transfer. Pigment Cell Res 18(5): 370-381, 2005.
  Comments: Study examining several independent approaches to establish assays to measure melanosome transfer between melanocytes and keratinocytes. They are summarized in Table 1 – none of them gave satisfactory results (perhaps with the exception of ^{14}C-thiouracil /see also *Dencker L et al /Acta pharmacol toxicol 49, 141-149, 1981/*).

- Chéhadé F, de Labriolle-Vaylet C, Moins N, Moreau MF, Papon J, Labarre P, Galle P, Veyre A, Hindié E.
  Secondary ion mass spectrometry as a tool for investigating radiopharmaceutical distribution at the cellular level: The example of I-BZA and ^{14}C-I-BZA. J Nucl Med 46: 1701-1706, 2005.
  Comments: Secondary ion mass spectrometry, exploited to study the tissue and cellular biodistribution of N-(2-diethylaminoethyl)-4-iodobenzamide (I-BZA), a compound targetting melanoma, revealed that I-BZA concentrated not only in the cytoplasm of both B16 melanoma cells and tumour infiltrating macrophages but also in the cytoplasm of normal melanocytes in the pigmented structures of skin and eye. EM demonstrated that melanosomes were responsible for I-BZA retention with a half-life of circa 38 hours.

- Chintala S, Li W, Lamoreux ML, Ito S, Wakamatsu K, Sviderskaya EV, Bennett DC, Park YM, Gahl WA, Huizing M, Spritz RA, Ben S, Novak EK, Tan J, Swank RT.
  ***Slc7a11* gene controls production of pheomelanin pigment and proliferation of cultured cells.** Proc Natl Acad Sci USA 102(31): 10964-10969, 2005.

Comments: *Slc7a* gene encoding plasma membrane cystine/glutamate exchanger was identified as a major genetic regulator of phaeomelanin production with minimal effects on eumelanin. Cells from *sut* mice (mutant in *Slc7a11*gene) were reported to have moderate deficiences of electron microscopy observable platelet dense granules, but in this study no changes in EM appearance of melanosomes were found. In *sut* melanocytes grown in vitro in the absence of ß-mercaptoethanol increased DOPA staining of tubular/vesicle structure (possibly the trans Golgi network) was noted unlike cells grown in the presence of ß-mercaptoethanol.

- Fowler DM, Koulov AV, Alory-Jost C, Marks MS, Balch BE, Kelly JW.
Functional amyloid formation within mammalian tissue. PLOS Biology 4(1): e6, 2006
Comments: Amyloid structure of melanosome fibrils was deduced from experiments based on the binding of dyes that fluoresce upon interaction with a cross-β sheet structures and on the ability to reconstitute Pmel17 amyloid formation *in vitro* as demonstrated by various biophysical techniques (X-ray fiber diffraction, CD and FT-IR spectra).

- Fukunaga M.
Perivascular epitheloid cell tumor of the uterus: Report of four cases. Int J Gynecol Pathol 24(4): 341-346, 2005.

- Hachiya A, Sriwiriyanont P, Kaiho E, Kitahara T, Takema Y, Tsuboi R.
An in vivo mouse model of human skin substitute containing spontaneously sorted melanocytes demonstrates physiological changes after UVB irradiation. J Invest Dermatol 125(2): 364-372, 2005.
Comments: Melanosome transfer to keratinocytes was correctly reorganized and melanin (melanosomes) evenly dispersed in the basal and suprabasal layers. The colour of human skin substitutes was found darker when cells of African descent were used in comparison to those of Caucasian origin.

- Immerstrand C, Nilsson MH, Lindroth M, Sundquist T, Magnusson KE, Peterson KH.
Height changes associated with pigment aggregation in *Xenopus laevis* melano-phores. Bioscience Rep 24(3): 203-214, 2004.
Comments: Melanosome aggregation in *X. laevis* melanophores resulted in a significant height increase across the cell centre, which was substantially larger when aggregation was induced by latrunculin than with melatonin. The elevation of the plasma membrane did not correlate with the influx of water through aquaporins or formation of new microtubules; rather the accumulation of pigment granules seemed to drive the change in cell height.

- Iyengar P, DeAngelis DD, Greenberg M, Taylor G.
Perivascular epitheloid cell tumor of the orbit: A case report and review of the literature. Pediatric Develop Pathol 5, 98-104, 2005.
Comments: A report of orbit PEComa showing on EM examination the presence of abnormal premelanosomes. Tumor cells were positive for HMB-45, tyrosinase, calponin and smooth muscle actin. Differential diagnosis between PEComa and melanoma, which can be pretty difficult, was discussed in detail.

- Levi V, Serpinskaya AS, Gratton E, Gelfand V.
Organelle transport along microtubules in Xenopus melanophores: Evidence for cooperation between multiple motors. Biophys J Oct 7, 2005 /Epub ahead of print/
Comments: The regulation of melanosome transport along microtubules was explored *in vivo.* The melanosome velocity depended linearly on the number of active motors. According to the authors 1 to 3 dynein molecules transport each melanosome in the minus end direction. The transport in the plus end direction was mainly driven by 1 to 2 copies of kinesin-2. The number of dyneins

transporting a melanosome increased during aggregation, while the number of active kinesin-2 stayed the same during melanosome aggregation and dispersion.

- Őstergren A, Svensson AL, Lindquist NG, Brittebo EB
  Dopamine melanin-loaded PC12 cells: a model for studies on pigmented neurons. Pigment Cell Res 18(4): 306-314, 2005.
  Comments: Cultured neuron-like PC12 cells were exposed to synthetic dopamine melanin. The cells took up the pigment granules. Their intracellular localization was similar to that in pigmented neurons *in vivo*. Dopamine melanin had negligible effects on the ultrastructure, on upregulation of endoplasmic reticulum stress and on activation of caspase-3 or cell viability. However, the EM evaluation was performed after 48 h which is too short period to observe any changes in resistant structures such as melanin and melanin moiety of melanosomes (*cf. Borovanský et al. / Folia biologica 45:47-52, 1999*).

- Sarangajaran R, Apte SP.
  Review: Melanization and phagocytosis: Implications for age related macular degeneration. Molecular Vision 11: 482-490, 2005
  Comments: A review on the mechanisms by which melanization by itself may contribute to a decrease in phagocytosis of outer rod segments and on the relationship between the rate of phagocytosis and rate of rod outer segments degradation in relation to lipofuscin formation.

- Schiaffino MV, Tacchetti C.
  The ocular albinism type 1 (OA1) protein and the evidence for an intracellular signal transduction system involved in melanosome biogenesis. Pigm Cell Res 18(4): 227-233, 2005.
  Comments: A stimulating review dealing with the OA1 protein, namely with its structure, biochemical features, subcellular localisation, disease-causing mutations and function. It seems that the OA1 might transduce information from the organelle lumen to the cytosol to regulate proper melanosome biogenesis and to inhibit organelle overgrowth.

- Theos AC, Tenza D, Martina JA, Hurbain I, Peden AA, Sviderskaya EV, Stewart A, Robinson MS, Bennett DC, Cutler DF, Bonifacino JS, Marks MS, Raposo G.
  Functions of adaptor protein AP-3 and AP-1 in tyrosinase sorting from endosomes to melanosomes. Mol Biol Cell 16(11): 5356-5372, 2005.
  Comments: Adaptor complexes AP-3 and AP-1 were shown to function independently in tyrosinase sorting from endosomes to melanosomes.

- Theos AC, Truschel ST, Raposo G, Marks MS.
  The *Silver* locus product Pmel17/gp100/Silv/ME20: Controversial in name and in function. Pigment Cell Res 18(5): 322-336, 2005.
  Comments: A review summarizing the structural and functional aspects of multialiased silver locus product that is involved in melanosome biogenesis, particularly in the formation of melanosome fibrillar matrix.

- Zhang H, Yang G, Chen H, Wei B, Ke Q, Guo H, Ye L, Bu H, Yang K, Zhang Y.
  Clinicopathological, immunohistochemical and ultrastructural study of 13 cases of melanotic schwannoma. Chinese Med J 118(17): 1451-1461, 2005.
  Comments: Melanotic schwanoma is composed of melanin-producing cells with ultrastructural features of schwannoma cells and can be misdiagnosed as melanoma. Both tumour types have been repeatedly found to be S-100, HMB-45 and MELAN-a positive. For their distinguishing immunohistochemical detection of laminin and collagen has been strongly recommended. Ultrastructurally, melanosomes in all developmental stages were observed in schwannoma cells and also a large number of melanosomes was demonstrated in macrophages scattered among tumour cells.

ANNOUNCEMENTS & RELATED ACTIVITIES

Calendar of events

2006 4th European Academy of Dermatology and Venereolog (EADV) Symposium

February 9-12, Saariselkä, Lapland, Finland
Contact : Ave General de Gaulle 38
B-1050 Brussels
Belgium
Phone: 32-2-650-00-90
Fax: 32-2-650-00-98
Email**:** office@eadv.org
E-mail: lapland2006@congrex.fi
Web: www.eadv.org/lapland2006

2006 1st Congress of the IDS (International Dermoscopy Society)

April 27-29, Naples, Italy
Contact : Giuseppe Argenziano, MD
Department of Dermatology
Naples , Italy
Email: giuseppe.argenziano@unina2.it
Website: www.dermoscopy-ids.org

2006 30th Annual Meeting of the Israel Society of Dermatology & Venereology

June 07-09, Tiberias, Israel
Contact : Ortra Ltd.-Conference Secretariat
Dr. Dganit Rozenmah
Dept of Dermatology
HaEmek Medical Center
Afula, Israel
Phone: 972-4-6494255
Fax: 972-4-6494120
Email: derm@ortra.com
Website: www.ortra.com/derm

2006 4th Summer Academy of Dermatopathology Meeting

July 31 - August 04, Graz, Austria
Contact : Department of Dermatology, Medical University Graz
Lorenzo Cerroni, MD
Auenbruggerplatz 8
8036 Graz , Austria
Phone: +43-316-385-2423
Fax: +43-316-385-2466
Email: lorenzo.cerroni@meduni-graz.at

2006 36th Annual ESDR Meeting

September 7-9, Paris, France
Contact: European Society for Dermatological Research
7 Rue Cingria
1205 Geneva, Switzerland
Phone: 41-22-321-48-90
Fax: 41-22-321-48-92
E-mail: office@esdr.org
Web: www.esdr.ch

2006 International Dermoscopy Course and Conference

September 7-9, Warsaw, Poland
Contact: Dept. Dermatology CSK MSWiA
Lidia Rudnicka
Woloska 137
00-768Warsaw, Poland
Phone: +48 22 824 22 00
Fax: +48 22 508 14 92
Email: lidiarudnicka@yahoo.com
Website: www.derm.pl

2006 13th meeting of the PanAmerican Society for Pigment Cell Research

September 7-10, Cincinnati, OH, USA
Contact: Zalfa Abdel-Malek
E-mail: paspcr13@uc.edu
Web : paspcr.med.umn.edu/

2006 Perspectives in Melanoma X

September 13-16, Amsterdam, Netherlands
Contact: Imedex
Phone: 770-751-7332
Fax: 770-751-7334
Email: meetings@imedex.com
Website: www.imedex.com

2006 6th Congress of BADV

September 14-16, Riga, Latvia
Contact: Andris Y. Rubins, MD
Riga Stadins University
Kr. Valdemara Street 76-75
Riga LV-1013, Latvia
Fax: 371-737-0395
Email: arubins@apollo.lv
Website: www.badv.lv

2006 XIIIth Meeting of the ESPCR

September 24-27, Barcelona, Spain
Contact: Dr. L. Montoliu
E-mail: montoliu@cnb.uam.es
Web: www.cnb.uam.es/~espcr06/

2006 15th Congress of the European Academy of Dermatology and Venereology - EADV

October 04-08, Rhodes Island, Greece
Contact: Mrs. Penelope Mitroyianni
Phone: 30-2-107-257-693
Fax: 30-2-107-257-532
E-Mail: info@eadv2006.com
Website: www.eadv.org

2006 XXVII Symposium of the ISDP

November 09-11, Malaga, Spain
Contact: ISDP - Cathy Klapak
PO Box 5717
Winston-Salem
NC 27113-5717 USA
Phone: 336-784-9156
Fax: 336-788-0742
Email: intsocdermpath@aol.com
Website: www.intsocdermpath.org

2006 20th Annual Meeting of the Japanese Society for Pigment Cell Research (JSPCR)

November 25-26, Matsumoto City, Japan
Contact: Prof. Toshiaki Saita of Shinsyu University
Web: wwwsoc.nii.ac.jp/jspcr/

2007 37th Annual ESDR Meeting

September 6-8, Zurich, Switzerland
Contact: E-mail: office@esdr.org
Web: www.esdr.ch

2007 14th meeting of the PanAmerican Society for Pigment Cell Research

September 13-16, Chicago, IL, USA
Contact: Caroline LePoole
E-mail: ilepool@lumc.edu
Web : paspcr.med.umn.edu/

2007 XIVth Meeting of the ESPCR

September, Bari, Italy
Organizer: Prof. Rosa Cicero

2007 21st World Congress of Dermatology

October 1-5, Buenos Aires, Argentina
Contact: E-mail: info@dermato2007.org
Web: www.dermato2007.org

2007 21th Annual Meeting of the Japanese Society for Pigment Cell Research (JSPCR)

December 8- , Toyoake City, Japan
Contact: Prof. Kazumasa Wakamatsu
Web: wwwsoc.nii.ac.jp/jspcr/

2008 20th International Pigment Cell Conference (IPCC)

May 7–12 Sapporo, Japan
Contact: Secretariat Office
Toshiharu YAMASHITA (Sapporo Medical University, Japan)
Minami 1-jo, Nishi 16-chome Chuo-ku, Sapporo, Japan 060-8543
Phone: +81-11-611-2111
Fax: +81-11-613-3739
E-mail: **mailto:ipcc-imrc2008@sapmed.ac.jp**
Web: **www.e-convention.org/ipcc-imrc2008/**

2008 International Investigative Dermatology (Joint Meeting of the ESDR, SID and JSID)

May 14-17 , Kyoto, Japan
Contact: E-mail: office@esdr.org
Web: www.esdr.ch

MINUTES OF THE ESPCR GENERAL ASSEMBLY

Wednesday, September 21, 2005, 10:30
IPCC Meeting
Lake Thoreau Room, Hyatt Hotel, Reston, VA, USA

1. Opening of the General Assemby.

The meeting was opened by J.-C. García-Borrón, who welcomed the 25 participants and all the members of the ESPCR Council but J. M. Naeyert.

2. Approval of the minutes of ESPCR General Assembly in Paris.

The Minutes of the ESPCR General Assembly in Paris were approved.

3. President's Report.

In the absence of the ESPCR Secretary, the classical secretary report could not be done. Moreover, the elections of officers were perturbed (see point #6). A short report was given by the President, who started by reviewing the legal status of our Society. Today, our organization is not legally registered, and a related issue is the lack of a VAT number for the Society. The VAT number is required for movement of money and for tax issues. Also, sponsors need a VAT number for donations, but this issue is unfortunately not important to day because we do not have any sponsor. Concerning payments to companies, a VAT number is now absolutely required to pay invoices such as those from Blackwell corresponding to *Pigment Cell Research* subscriptions. Transitory solutions are possible through the IFPCS, and the help and availability of IFPCS Officers was acknowledged. Concerning taxes, this is not a real problem because the ESPCR income is too low to be taxable. The President proposed to leave the VAT issue to the next ESPCR President, and this was accepted.

4. Treasurer's Report.
This was delivered by Jo Lambert.

- Statistics
The number of members in the ESPCR Web Site List is 181. However, the actual number of members in good standing is only 121 (8 honorary members, 91 regular members, 22 student members). We have 36 new members. This increase can be due to a better welcome package, the benefits of becoming an ESPCR member and the price of IPCC meeting for members and non-members.

- Finances
The finances are good over the year, a positive balance was produced: 1596.01 euros
A detailed list of income and outgoings follows:

Income 10/2004-09/2005	
Member subscriptions 2004	6120
Pigment Cell Research subscriptions	4150
Donations	80
Donations from IFPCS (= US $ 4000)	3320
Balance carried over from 2004:	1758.79
Total income (1758,79+ 13670)	**15428.79**

Outgoings 9/2004-9/2005

IFPCS subscriptions for 2005 (91 x $28 = $ 2548)	2114.84
ESPCR Meeting + Fritz Anders Memorial Lecture	0
Bulletin and web costs	210.00
Bank charges Centea	165.40
Credit card machine (running budget)	545.63
Membership VSDC (advise organ VAT issue)	095.00
ESPCR Travel Awards	2000.00
Pigment Cell Research Subscriptions (35 x128)	3718.40
Pigment Cell Research Subscriptions (28 x128)	2974.72
Best poster prize ESPCR 2004 Paris (Moriyama)	250.00
Total outgoings	**12073.99**
Balance on 21/09/05	**3354.80**

The report was approved by the General Assembly, and the President thanked Jo Lambert for her work.

5. ESPCR Bulletin and Web site report.

- Web site

G. Ghanem reported that the Web site has been regularly updated and improved. Concerning the Bulletin, each time a new issue is announced, there are about 800 hits to the web site. This is very encouraging and means that the bulletin is read by many people and is useful. The persons involved in the bulletin were thanked.

6. Forthcoming elections

Major elections need to be conducted in the near future. The Officers Elections are due before the end of 2005. Early in 2006, Council Elections should be carried out to cover a large number of positions.

- Officer Elections:

The three officers should have been elected before this meeting. J. M. Naeyert was nominated as President, Lionel Larue as Secretary and Anja Bosherhof as Treasurer. Unfortunately, this process was postponed, due the health problems of J. M. Neayaert. The Council decided to open a new call for nominations for the three officers. It has to be pointed out that the nomination of Lionel Larue as Secretary and Anja Bosherhof as Treasurer were declared and remained valid.

The new call for nominations will be issued on October 1st by the acting secretary, Lionel Larue, using the email/fax procedure. An encouraging letter has to be written to get new candidates and defining the charge of each officer.
On November, nominees for each charge will undergo a ballot if required.
On December 1st, the results will be announced, and the elected officers will take on their duties on September 2006.

The President raised the issue of a possible change of the Constitution to improve certain aspects of the Officers elections process. According to the current Constitution, Officers are elected for a three year term and cannot stand for two consecutive periods. However, in the case of the Treasurer, this causes extra and unnecessary expenses as the bank account follows the Treasurer. Moreover, this introduces a further degree of complication for the VAT issue. Therefore, it was proposed that the Treasurer could run for two consecutive 3 year terms. The President and the Secretary will continue to run only once. This motion was adopted unanimously. The bylaws will be modified accordingly.

- Council Elections:
Concerning the Elections for Council, a call for nominations will be performed by email by the Secretary in January 2006. The election will be performed immediately after, and the elected members will effectively join the Council during the second Council Meeting to be held in the next ESPCR Meeting, in Barcelona, September 2006.

7. Venues for forthcoming Meetings.

The venue of the 2006 XIIIth ESPCR Meeting is Barcelona, Spain, organized by L. Montoliu.
The venue of the 2007 XIVth ESPCR Meeting is Bari, Italy, organized by Profsa. R. Cicero.
The venue of the next IPCC 2008 Meeting is Sapporo, Japan, organized by Prof. K. Jimbow.

The venue of the 2009 XVth ESPCR Meeting has to be decided. Several possibilities were mentioned, including Bath (R. Kelsh), Münster (M. Böhm), Geneva (B. Wehrle-Haller) and others. Prof. T. Sarna mentioned his possible interest to organize the meeting in Poland. It was decided that the Officers will discuss all this issue with potential organizers and pass any news to the Council for decision. There is no pressure for the time being.

8. Any other business

Prof. Westerhoff is becoming an Honorary member of our Society, following a unanimous Council decision. This nomination was congratulated and applauded.

9. Close of the Meeting.

With no other matters to discuss, the meeting was closed by J.-C. García-Borrón who thanked all attendants.

ADDENDUM

The composition of the ESPCR Council is:

OFFICERS
President: J. C. García-Borrón (Murcia)
Secretary: J.M. Naeyaert (Ghent)
Treasurer: J. Lambert (Ghent)

COUNCIL
F. Beermann (Lausanne)
Dorothy Bennett (London)
G. Ghanem (Brussels)
C.R. Goding (Oxted)
L. Larue (Paris), acting as Secretary
M. Picardo (Rome)
N. Smit (Leiden)
A. Taïeb (Bordeaux)

Election of ESPCR Officers, 2006-2009

Following a call for nominations issued in October, only one candidate has been nominated for each of the three ESPCR Offices by the closing date.

It is a great pleasure to announce that the three nominees are highly talented, experienced and dedicated individuals who will fulfil these posts very effectively. We take this opportunity to thank them very warmly for being prepared to serve our Society, and to wish them the best luck for their term. Their terms of office will begin in September 2006. Their names are:

President: Prof. Mauro Picardo (Italy)

Secretary: Dr. Lionel Larue (France)

Treasurer: Prof. Jo Lambert (Belgium)

NEW MEMBERS

The ESPCR is delighted to welcome the following colleagues to membership and hope they will play a full and active part in the Society

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