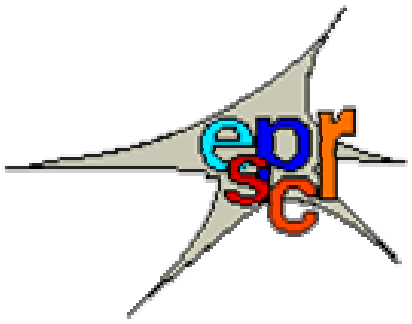
N° 47 Dec 2003

E S P C R B U L L E T I N

PUBLISHED BY THE EUROPEAN SOCIETY FOR PIGMENT CELL RESEARCH



EUROPEAN SOCIETY FOR PIGMENT CELL RESEARCH *BULLETIN*

N° 47 - Dec 2003

Editorial Office: *G. Ghanem (Editor), C. Meunier, R. Morandini (Production Team), Laboratory of Oncology and Experimental Surgery (L.O.C.E.), Université Libre de Bruxelles, Institut J. Bordet, Rue Héger-Bordet 1, B – 1000 Brussels, Belgium.*
Phone: 32-2-541.32.96 Fax: 32-2-534.95.50 E-Mail:gghanem@ulb.ac.be

*** HAPPY NEW YEAR 2004 ***

CONTENTS

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

A MESSAGE FROM PROF. JOSÉ CARLOS GARCÍA-BORRÓN
NEW ESPCR PRESIDENT

Dear Friends and Colleagues,

As every year, it was a real pleasure to meet many of you in September, this time in Ghent during the XIth ESPCR Meeting. This was a special meeting for me in many aspects, and among them because I started it as the ESPCR secretary, and finished it as president. It was also a cheerful occasion to see so many old friends again, and to make new ones. The ESPCR has many things to offer, and the friendly atmosphere of its meetings is certainly not the least important.

However, on writing this letter, the one thing that first comes to my mind is certainly not a happy memory, but the sadness for the loss of Prof. Giuseppe Prota, who passed away January 10, 2003. When, stirred by Giuseppe, the Society was established in 1985, he became the Chairman of the Interim Council, and was elected Inaugural President at the first ESPCR Meeting held in Sorrento in 1987. Giuseppe was, no doubt, the principal person responsible for the birth of our Society. But, much more than this, from the very beginning of the ESPCR he remained an example to follow and a source of inspiration for the pigment cell community. I am sure that many of you missed his sparkling conversation and sharp comments in Ghent as much as I did, and could not help but experience a sense of profound loss. I will not try to summarize his scientific achievements and key contributions in various areas of pigment cell research, since this has already been done by others. But, on behalf of Giuseppe's many friends, I want to take this opportunity to express our deep gratitude to all of those who contributed to honour his memory. In particular, thanks to Profs. Patrick Riley and Marco d'Ischia for their poignant In Memoriam writings, to the Editor-in-Chief of Pigment Cell Research, Prof. Vince Hearing, for his enthusiasm in setting up a Special Commemorative Issue, and to Marco d'Ischia and Mauro Picardo who served as Guest Editors. Last, but not least, thanks to Prof. Ghanem Ghanem, for the contribution of the ESPCR Bulletin to the memorial activities, and to Profs. Jean Marie Naeyaert and Jo Lambert who, as Organizers of the Ghent Meeting, succeeded in putting together a memorable "G. Prota Session on Chemistry of Melanins". This session provided an excellent opportunity to appreciate not only the depth of Giuseppe's contribution, but also the vitality of the Naples School initiated by him.

As the ESPCR moves quickly to its twentieth anniversary, I must also acknowledge sincerely the work of those who have contributed to its present strength. Since the list is already long and impressive, I will just express here our gratitude to the outgoing Officers, Prof. Dorothy Bennett and Dr. Lionel Larue. I am sure that all of you know them as leading scientists in pigment cell research, and as pro-active members of our Society, always ready to collaborate, and to exchange ideas. But many of you may not be aware of their warmness and their fine, and complementary, sense of humour. To work with them during the last three years has been a privilege, but also, it has been fun. I am happy that they remain on the Council, and that they will, no doubt, keep on contributing to make the running of our Society more open, simple, interactive and efficient. And this brings me to the new team of Officers. Here again, the input and enthusiasm of the Ghent group must be underlined. In addition to setting up the wonderful XIth ESPCR Meeting, Jean Marie Naeyaert and Jo Lambert have been elected as Secretary and Treasurer. The least I can say is very many thanks for their availability. For many years, Ghanem Ghanem and his team have been running efficiently and successfully the ESPCR Bulletin and the ESPCR Web site, and deserve here a special mention. Ghanem is always ready to receive any comments or suggestions, and to work hard to improve those "windows" of the ESPCR. His work and availability deserve the most sincere acknowledgement.

It is difficult to imagine a better setting for the 2004 ESPCR Meeting than Paris, and a better Organizer than Lionel Larue. Therefore, I am looking forward to meeting most of you there. And I also hope to get to know many new members. Accordingly, I would urge senior members to encourage potentially interested scientists, particularly their students, to join the Society and attend the Paris Meeting. They have much to learn and many interesting people to meet there. Clearly, the value return for a student's subscription is excellent, including eligibility for travel grants and free Pigment Cell Research subscriptions, and access to the Bulletin where you are reading these words.

One of the main assets of our Society is the easy, friendly and productive interaction between members, and the rapid feedback between Officers, Council and membership. Should you need any information, or wish to make any comments or suggestions, please do not hesitate to contact me, or any of my fellow Officers or Council Members. Contact details are available through our Web site. The more we hear from you, the more our Society will reflect our common interests, and the more congenial and productive its life will be.

This is an excellent opportunity to send all of you my seasonal greetings, and to wish you a happy New Year, on behalf of ESPCR and its Council.

Looking forward to seeing you in Paris.

José Carlos García-Borrón
President, ESPCR

A MESSAGE FROM THE PRESIDENT OF THE INTERNATIONAL FEDERATION OF PIGMENT CELL SOCIETIES, TO MEMBERS OF THE ESPCR, JSPCR AND PASPCR <WWW.IFPCS.ORG>

Dear Friends and Colleagues,

Seasonal greetings, and a happy and peaceful New Year to all of you, on behalf of IFPCS and its Council. 2003 may have been another disturbing year in world politics, but at least it has seemed a good year for the Pigment Cell Societies and their research. Successful regional meetings were organized for the ESPCR by Jean-Marie Naeyaert and colleagues, in historic Ghent (Belgium), and for the PASPCR by John Pawelek, Jean Bolognia and their team in scenic Cape Cod (Massachusetts). At the time of writing, all is set for the JSPCR meeting, to be chaired by Genji Imokawa in cosmopolitan Tokyo. As usual, anyone unable to attend these meetings can find the abstracts published as supplements to issues of the journal *Pigment Cell Research*. For a different perspective, you can find some pleasant photographs of the ESPCR and PASPCR meetings, on their Society web sites.

News from the IFPCS: In 2003, Lionel Larue completed his term on IFPCS Council, and we thank him warmly for his active and valuable participation. In his place, we welcome Jean-Marie Naeyaert, the new ESPCR Secretary. Progress continues apace with the organization of the 19th International Pigment Cell Congress, September 18-23, 2005, to be chaired by Vince Hearing (NIH, Washington) for PASPCR. The IPCC is the triennial meeting of our Federation, where all the member societies meet together. Please help to pass on news of this essential meeting to all researchers interested in this field. Planning is already well developed, with prospects of generous sponsorship through the successful advocacy of Dr Hearing and colleagues. The congress will not now be held in the NIH as envisaged, for good reasons including a large price increase, but will be in a congenial venue not far away, the Hyatt Regency Hotel, Reston. You can check the web site, <ipcc.info>, for regular updates. Looking even further ahead, it is a pleasure to announce that the 20th IPCC in 2008 will be organized by the JSPCR in Sapporo, and will be chaired by Prof. Kowichi Jimbow.

Meanwhile, in 2004, please support your regional Pigment Cell Society conferences, and help to make these the scientifically broad and excellent, yet friendly and congenial, occasions we have come to expect. These will be in Newport Beach, California (PASPCR) in June, Paris (ESPCR) in September and Kumamoto (JSPCR) in November, 2004. Please see the society web sites for information, or contact the organizers, respectively: Dr Frank Meyskens <flmeyske@uci.edu>, Dr Lionel Larue <Lionel.Larue@curie.u-psud.fr> and Prof Tomomichi Ono (contact Dr Kageshita, Secretary-General, <toshiro@kaiju.medic.kumamoto-u.ac.jp>). Please come, and please also encourage colleagues who are not yet society members to join your regional society and take part in these first-rate and stimulating conferences. IFPCS Council will meet next in Kumamoto, so we in the Council look forward to meeting many members of the JSPCR in 2004.

Our IFPCS journal *Pigment Cell Research* goes from strength to strength, with further increases in subscription numbers and Impact Factor (now 2.2), as Vincent Hearing approaches the final year of his impressively successful term as Editor-in-Chief. From January 2005 we will be welcoming a new Editor-in-Chief, Dr Colin Goding (UK), and I am confident that he will take up the big challenge left by Vince's achievements, and will continue the journal's progress. Increasing readership and impact, more colour pages and continued rapid turnover of submitted manuscripts are just a few of the good reasons to submit your own work to *PCR*. Don't forget that you can also support the journal by citing recent *PCR* articles, and by encouraging your library and colleagues to subscribe to the journal. During 2003, the journal has also published two special issues: the Proceedings of the 2002 IPCC, and an issue commemorating the sad death of Giuseppe Prota, principal founder of ESPCR and past President of IFPCS and of ESPCR. This event was also commemorated at the ESPCR meeting, with a special dedicated session and a talk by Marco d'Ischia in memory of Prof Prota and his exceptional contributions. Returning to *PCR*, we continue to be indebted to *PCR*'s generous corporate sponsors Johnson & Johnson, L'Oréal, Shiseido and Unilever, who have all recently agreed not only to continue but to increase their level of annual support. This should enable the IFPCS to maintain the numbers of free *PCR* subscriptions it can provide, for the societies to allocate to their younger (or other deserving) members.

More information about the IFPCS, and its various components and interest groups, can be found at the IFPCS web site, <www.ifpcs.org>, ably maintained by Dr Bill Oetting. There are links to various related sites and resources. Note for geneticists: the IFPCS Mouse Coat Color Genes page, <http://www.cbc.umn.edu/ifpcs/micemut.htm>, has been extensively updated and enlarged this year to include all of the 130 or so known, mapped mouse pigmentary loci (cloned and uncloned). A particularly useful feature is that gene listings are directly hyperlinked not only to their entries in the Mouse Genome Database, but also to those in Online Mendelian Inheritance in Man (OMIM) for the corresponding human genes.

The IFPCS Special Interest Groups or SIGs have continued to be active - see <www.ifpcs.org>. The former IFPCS Melanoma Interest Group, chaired by Meenhard Herlyn, <herlynm@wistar.upenn.edu>, has now transformed into a full international society, the Melanoma Research Society. They held an outstanding Melanoma Research Congress in Philadelphia in June 2003, and will meet again in Arizona in November 2004. We wish the new society every success. The IFPCS Pigment Cell Development Group is planning another workshop at the NIH in April 2004; information from its chair Bill Pavan (NIH) <bpavan@nhgri.nih.gov>. All are welcome to join the group (no cost) or attend the workshop

(some cost). The IFPCS Pigment Cell Genetics Group is about to re-form, under its new chair Ian Jackson (Edinburgh) <ian.jackson@hgu.mrc.ac.uk>, and a first provisionally planned activity will be a workshop at the 2005 IPCC. Lastly - not exactly an interest group - the IFPCS Women Scientists' Committee has also been reformed. This committee is available if needed, to advise or help women or minority scientists who are IFPCS society members. The new members are Dr Estela Medrano (PASPCR representative and chair), Prof. Paola Grammatico (ESPCR) and Prof Noriko Oshima (JSPCR). See <www.ifpcs.org> for their contact details.

In conclusion, I hope that you will take a look at the numerous IFPCS activities and resources, and will find something valuable. Any suggestions for further activities or improvements will always be welcomed – please contact me at <dbennett@sghms.ac.uk>.

Once again, best wishes to everyone for a happy and successful 2004.
Dot Bennett
[President, IFPCS]

Meeting Report
XITH MEETING OF THE ESPCR
17-20 sep 2003 – GHENT, BELGIUM

INTRODUCTORY REMARKS

It was with an immense pleasure and pride that we hosted the 11th Meeting of our Society in our hometown GENT in Flanders. After a welcome reception in the town hall on Tuesday evening, the scientific meeting started on Wednesday in the great AULA of Ghent University with introductory remarks from both the Rector of the University and the Dean of the Faculty of Medicine. In all, thirteen plenary symposia followed, touching all aspects of pigment cell research in a multidisciplinary fashion, one of the great strengths of our Society. The Fritz Anders Memorial Lecture "The genetics of susceptibility to melanoma" was delivered in a remarkable way by Dr Julia Newton-Bishop. In order to commemorate the great pioneer in melanin research, Giuseppe PROTA, a G. PROTA session on Chemistry of Melanins was created and will continue to be a structural part of future meetings. The talks during this session were delivered by disciples of the Naples School and close friends of G. PROTA.

The Special Lectures were enjoyed by all present, since they were delivered by top scientists in various fields such as proteomics, immunology, genetics and melanogenesis. The introduction of scientists not working in pigment cell research was a deliberate choice and worked out greatly creating new ideas for future research. More than 30 invited lectures covered all fields in a most exciting way, many of them using spectacular videos and/or digital pictures.

Continuing the tradition set in Rome, a more clinically-oriented satellite symposium was organised on Saturday, coinciding with the autumn meeting of the Royal Belgian Society of Dermatology. Epidemiology, genetics, autoimmunity and treatment of vitiligo were highlights of the morning session, while the afternoon covered more clinically oriented items.

The gala dinner on Thursday evening in the Ooidonk Castle was an unforgettable event for many and cocktails were enjoyed on the grounds of the castle with –for Belgium!- exceptional sunny weather.

Looking back at the scientific work that was presented during this meeting, one can only conclude that the ESPCR is thriving and that the scientific quality of the work done in many laboratories is steadily increasing.

We hope all attendants enjoyed the programme and the city of Gent and look forward to see you back in Paris next year!

Jean Marie Naeyaert, Conference Organizer, and
Jo Lambert, Conference Secretary, on behalf of the Organizing Committee.

PLENARY SYMPOSIA

Plenary Symposium I: Biochemistry of melanogenesis

Chairs: F. Solano, H. Rorsman

Contributed by F. Solano (Murcia)

The symposium I was opened by Dr. Vince Hearing, who offered to the attendants an integral view of the melanosome, its protein components and the way they are involved in the regulation of the melanogenesis, movements on the dendrites and transfer to neighbouring keratinocytes. Dr. Hearing and his collaborators have performed a superb proteomic analysis of the melanosomes and they have characterized about 70 different proteins in those organelles. Concerning the well-known enzymatic proteins, tyrosinase and its family, the last advances are related to the sequence of events in which those enzymes are delivered to melanosomes, mostly at early stages, I and II. Using two antibodies against the silver protein (gp100), Pep13 and HMB45, directed against different epitopes, Dr. Hearing reinforced the early data pointing out that gp100 should be cleaved in its C-terminal tail to become the structural fibrillar component of the eumelanosomes.

Aside these proteins as classical regulators of melanogenesis, melanosomes contain some ATPases, in particular vATPase, which can regulate the intramelanosomal pH and thus the catalytic function of the above mentioned melanogenic proteins, mostly tyrosinase as the key enzyme for melanin formation. Some differences in the intramelanosomal pH could be the reason of the different melanogenic activity in the black and white skins.

Concerning the regulation of the melanosome motility and transfer to neighbouring keratinocytes, melanosomes contain a number of common components with other related membrane-bound organelles such as lysosomes and endoplasmic reticulum. Some of them are related to the biogenesis of the melanosome and others to the fate and further movements once they matured and should migrate to the dendrites and finally exported. To these regards, Dr. Hearing presented some data about Rab proteins that are involved in the intracellular motility and transfer of melanosomes.

The next speaker to give the second invited lecture was Prof. García-Borrón, whose talk was entitled "Biochemical Aspects of MC1R signalling through the cAMP pathway". Prof. García-Borrón offered an updated view of the melanocortin 1 receptor and its mechanism for the signal transduction mediated by heterotrimeric G proteins. He underlined the differences between the mouse and human receptors concerning affinity for melanocortin ligands and the density of receptor molecules in the membrane of both types of cells. This is important since mouse is usually used as a model animal to extrapolate data to human, and it is now well established that mutations in the melanocortin 1 receptor are associated with skin phenotype and with an altered risk of melanoma.

To perform that study, he used a panel of melanoma cells and heterologous systems for the transient or stable expression of the cloned genes of MC1R (human) and Mc1r (mouse), and then several aspects of the functional coupling to the cAMP pathway were analysed. First, he showed that MC1R has much lower density compared to Mc1r but the human species displays a strong agonist-independent constitutive activity in comparison to the mouse receptor. This activity should be related to the constitutive acquisition of an active state in the human receptor in absence of any ligand. This should occur of in spite of the high homology (around 80% between both proteins), and the residues or regions of the molecule responsible of that big difference is under study. However, the kinetics for desensitization was similar in both species. Going further, he also showed that desensitization was mediated by a family of kinases for the receptors associated to G proteins (GRKs). In accordance to the similar kinetics, mouse and human showed the same types of GRKs, particularly GRK2 and GRK6. Overall, this lecture offered the audience new updated insights on the activation of MC1R, its coupling to the cAMP signal transduction pathway and the regulation of its desensitization.

After those two invited lectures, the symposium continued with two free communications. The first one was given by Dr. Kasraee, from the Univ. Hospital of Geneva, in Switzerland, who presented some data concerning the formation of melanin in extracutaneous cells lacking tyrosinase (neurons, phagocytes, macrophages, etc.). Under this enzyme-missing condition, he investigated the mechanism to oxidize tyrosine until melanin. Rather than an enzymatic mechanism, he proposed a chemical one: hydrogen peroxide plus some intracellular antioxidant, such as ascorbic acid, GSH or NADH are able to carry it out. In that way, melanin production in extracutaneous cells could be considered a defence mechanism to protect those cells against the oxidative stress associated to generation of hydrogen peroxide by consuming this oxidant.

The last free communication was presented by Dr. C. Jimenez-Cervantes, from the Faculty of Medicine in the Univ. of Murcia, Spain, who presented a detailed study of the constitutive activity of the human melanocortin 1 receptor. She analyzed the agonist-independent signalling of the human and mouse receptors expressed in some heterologous cell lines, such as HEK293T or CHO, and in several human melanoma cells. She found some interesting differential agonist-independent responses between expression of the human and the mouse receptors, in terms of intracellular cAMP increase. At similar receptor densities, the increase in cAMP is much higher for the human than for the mouse protein. In addition, the cAMP increase was not observed for several alleles of human receptor with point mutations. Therefore, she concluded that human MC1R has some constitutive activity. This could be related to its low expression in human melanocytes and the effects of the agouti signalling protein.

Plenary Symposium II: Developmental biology of pigment cells

Chairs: E. Dupin, A. Thody

Contributed by J.C. García-Borrón (Murcia)

Plenary Symposium II was devoted to the developmental biology of pigment cells and included three invited lectures and an oral communication, that dealt with the function of key signalling molecules and their signalling pathways. The first invited lecture, entitled “The role of Kit-ligand presentation in the development of the epidermal melanocyte niche”, was presented by B. Wehrle-Haller, from Geneva. The key role of the kit-ligand (also called Stem Cell Factor or Steel Factor) on the proliferation, survival and migration of embryonic melanoblasts has been known for years. But the data presented by Wehrle-Haller introduced a new aspect derived from the recent characterization of the signals required for the traffic of the kit-ligand at the basolateral membrane of epithelial cells. This knowledge, together with a cellular co-culture model comprised of kit-ligand responsive melanoblast cells and kidney epithelial cells allows to study the role of correct kit-ligand presentation towards melanoblasts. One interesting conclusion, that may have implications for our understanding of melanoma progression, is that melanoblasts survive correctly in the basolateral side of kidney epithelial cell monolayers expressing the wild type kit-ligand, but migrate from this monolayer when confronted to an “apical” presentation of misrouted kit-ligand missing a basolateral targeting sequence.

The second invited lecture was delivered by E. Steingrimsson (Reykjavik), who spoke on the *microphthalmia* transcription factor network. This basic-helix-loop-helix-leucine zipper transcription factor regulates the cell-specific expression of target genes, and has been shown to interact with various partners, including TFE3, TFEB and TFEC. Steingrimsson’s group looked for new partners of *microphthalmia*, using a yeast-two hybrid approach. Among other partners, the Mitf protein was shown to interact with p66, a protein participating in a histone deacetylase/DNA methylase complex. This raises the possibility that Mitf activity might be regulated by acetylation.

The last invited lecture of the symposium was given by L. Larue (Paris), who presented data on the effects of β-catenin overexpression on the migration and differentiation pattern of melanoblasts. The study reported was performed using transgenic mice models expressing mutant forms of β-catenin. β-catenin plays a pivotal role in the Wnt pathway, which is important for melanocyte growth and differentiation and is frequently disregulated in melanoma. Wnt signalling results in the stabilization of β-catenin, which then translocates to the nucleus. In the nucleus, β-catenin cooperates with members of the Lef/Tfc family of transcription factors to upregulate expression of *Microphthalmia*. A similar stabilization with translocation to the nucleus occurs constitutively in the absence of Wnt signalling for β-catenin mutants lacking serine residues located in the N-terminal portion of the molecule, and likely targets for phosphorylation. Transgenic mice overexpressing those constitutively stable catenin mutants displayed several pigmentation phenotypes, including unpigmented belly spots and an overall decrease in general pigmentation. These data, which are interpreted in terms of impaired migration and proliferation of melanoblasts, underscore the importance of β-catenin signalling in normal development of the melanogenic system.

The session was closed by C. Real, from Nicole Douarin’s laboratory (Nogent-sur-Marne), who presented evidence for the *in vitro* reversal of melanocytes into self-renewing multipotent cells. During embryonic development,

multipotent neural crest stem cells differentiate into several cell types, including neurons, glial cells, smooth muscle cells and melanocytes, among others. Using cell type specific markers, it was demonstrated that clonal expansion of quail pigment cells in the presence of endothelin 3 gave rise to three types of clones: myofibroblastic-glial-melanocytic (FGM), glial-melanocytic (GM) and melanocytic (M), indicative of the reversion of melanocytes to multipotent cells. Self renewal of multipotent cells was suggested by subcloning assays, where FGM and GM clones were found present after several subcloning rounds. Interestingly, analysis of HNK1 expression, a marker of undifferentiated neural crest cells, showed that individual melanocytes gave rise to cells expressing this marker, and therefore similar to neural crest-like precursors. It was concluded that melanocytes are able to reverse their phenotype, at least when stimulated by the mitogenic endothelin 3.

Plenary Symposium III: Signalling pathways in melanocyte differentiation

Chairs: B. Gilchrest, S. MacNeil, E. Steingrimsson
Contributed by S. MacNeil (Sheffield)

The underlying theme of this symposium which contained an invited lecture by Colin Goding and three short communications was two fold – what intracellular signalling pathways are involved in normal melanocyte differentiation and how can melanoma cells behave so differently to melanocytes – can specific changes in their intracellular signalling machinery be identified?

Firstly, Colin Goding from the Marie Curie Research Institute in the UK reported on work from his laboratory dissecting which genes matter most to melanocyte development. This laboratory for a number of years has focussed on the role of *Microphthalmia* transcription factor (Mitf) in regulating melanoblast survival and activation of pigmentation genes. Upstream and downstream of Mitf are transcription factors which regulate the expression of Mitf. In particular Goding's group reported on a transcription factor Brn-2 upstream from Mitf which is highly over expressed in melanoma and TbX-2 which lies downstream from Mitf which has a role in organisation of chromatin during cell division and has anti-senescent activity.

Changes in the level of transcription factors which regulate or are effected by Mitf could alter the cell's sense of "self" and coupled with anti-senescent activity could be good news for melanoma progression and bad news for normal melanocyte differentiation.

A second talk on Mitf from Khaled et al from Nice in France looked at the role of Mitf in melanogenesis in mouse B16 melanoma cells. Again Mitf was found to play a key role in melanocyte survival and differentiation and work on the B16s suggested that it is in turn controlled at a transcriptional level.

Interestingly Mitf was found to be a convergence point in the cell in that melanogenesis can be increased either by elevating cyclic-AMP and activating the PKA-CREB pathway leading to the stimulation of Mitf or stimulated by inhibiting phosphatidylinositol-3 kinase (Pi3K) which increases the intracellular content of Mitf . Thus there appears to be two possibly related routes to increase Mitf. The complexity of this signalling system as it emerges strongly suggests that the control of melanocyte differentiation and melanogenesis is a highly regulated business for the melanocyte and far from simple.

The next two presentations focussed on how melanoma cells differ from melanocytes, Gaggioli et al from Nice in France looked at the role of hepatocyte growth factor/scatter factor (HGF/SF) fibroblast derived cytokines that acts as a mitogen for human melanocytes.

In looking at how murine B16 melanoma cells respond to HGF/SF, an early growth response factor 1 (Egr-1) was identified as being rapidly and transiently induced by HGF. However, Egr-1 expression is not induced in normal melanocytes in response to HGF. This raises the question of how melanocytes and melanoma cells differ in their response to HGF and stimulation of Egr-1 expression and the group are currently focussing on the function of Egr-1 in melanoma cells. Their data indicate that the RAS/RAF/MEK/ERK mediated pathway is involved in Egr-1 expression and that this responsiveness of the cell to HGF/SF might be associated with tumour progression.

Plenary Symposium IV: The G. Prota session on chemistry of melanins

Chairs: A. Napolitano, F. Solano
Contributed by A. Napolitano (Naples)

The IV plenary symposium dedicated to Professor Giuseppe Prota, was a momentous event of the XI ESPCR meeting which afforded the opportunity to the many close friends and colleagues for a scientific and sentimental tribute to his memory. As recalled many times during the meeting, and by professor Naeyaert in the opening speech, professor Giuseppe Prota was one of the founding member of the European Society for Pigment Cell Research and the first President. He was also the President of the International Federation and received all the major awards of the pigment cell community: the Myron Gordon award, the Memorial Seiji Lecture, the Raper Medal, the melanoma award from the WHO. He was the author of the classic textbook on Melanins and Melanogenesis, and was one of the executive editors of the international journal Melanoma Research.

All the lectures of the session were focused on issues which occupied most of Prota's research interests. The memorial lecture entitled "Melanins and melanogenesis: the changing landscapes. A tribute to Giuseppe Prota." was held by Marco d'Ischia, his closest collaborator, who guided us in an ideal journey through Prota's research activities from the early studies in the sixties to the recent works at the beginning of the new millennium. What appeared clear from the lecture was that at the time when Prota started his studies the field was very obscure as summarized by an original sentence by Blois according to which melanins were the most enigmatic pigments found in nature, and melanogenesis was little more than a

short sequence of poorly defined events. Prota's work on the chemistry of melanogenesis led eventually to a reformulation and expansion of the Raper-Mason scheme of melanogenesis, and provided experimental evidence which disproved many deeply rooted concepts. The rearrangement of dopachrome and the oxidative polymerization of 5,6-dihydroxyindoles were two critical steps of the biosynthetic route which were fully elucidated leading to a reappraisal of the role of 5,6-dihydroxyindole-2-carboxylic acid and definition of the indole positions involved in the polymerization process. It was also emphasized that the discovery of the cysteinyldopas and the swithching of the eumelanin pathway to pheomelanin formation was probably the most important achievement of Prota's work. In the 90's, characterization of post-synthetic structural modifications of melanins provided a key for the interpretation of the variety of natural pigments while the wide range of properties of diffusible melanogens suggested new functional roles in addition to pigment formation. The conclusion was that these advances contributed to a radical changing of the panorama of melanogenesis which is nowdays regarded as more significant that the mere provision of pigments and is actually perceived as a complex fine regulated process which plays a range of roles central to skin homeostasis and functioning.

The lecture stimulated a lively debate to which Vince Hearing, Patrick Riley and others significantly contributed. The regulatory mechanisms of melanogenesis, the biochemical factors responsible for the switching between the eumelanin and pheomelanin pathways and how these concepts might be reconciled with the activity of melanocortin receptors were addressed in the discussion.

The chemistry of pheomelanogenesis was the focus of the following lecture which presented an overview of *in vitro* studies on the reactivity of the biosynthetic intermediates with special emphasis on 1,4-benzothiazines. These were obtained mainly by oxidative cyclization of the primary pheomelanin precursor 5-*S*-cysteinyldopa. A systematic investigation of the oxidation of 5-*S*-cysteinyldopa under biomimetic conditions was presented which unraveled new reaction routes of the pheomelanin pathway including rearrangement and redox exchange processes leading to carboxylated/decarboxylated benzothiazines or dihydrobenzothiazines depending on a fine modulation of the reaction conditions. It was also pointed out how the recent advances in analytical and spectral methodologies had allowed identification of previously unknown very fugitive intermediates, characterization of oligomer species along the oxidative polymerization pathway toward the final pheomelanin pigment and even direct analysis by mass spectrometry of synthetic pheomelanins in comparison with the natural pigment isolated from human hair. A likely biogenetic route was also postulated for the $^{2,2'}$-bi(2*H*-1,4-benzothiazine) pigments, termed trichochromes, whose origin had remained an open issue since their first isolation from red human hair and avian feathers in the seventies. Overall, the presentation provided a rather detailed description of the structure of pheomelanin pigments in terms of the monomer units, their mode of linking and post-synthetic modifications. In the following debate the possibility was discussed that the newly identified benzothiazines intermediates might be used as biochemical markers of the pheomelanin pathway in addition to 5-S-cysteinyldopa; yet, the limitations of such an approach due to the inherent instability of these intermediates were clearly pointed out.

In the last proffered paper of the session Anna Palumbo summarized biochemical studies carried out in the last two decades on the melanogenic enzymes of the ink gland of *Sepia officinalis* generally recognized as a most convenient model for studies of melanogenesis. It was shown how some of these enzymes have a peculiar activity different from that observed in other organisms. A noticeable example is the dopachrome rearranging enzyme which catalyses the rearrangement of dopachrome to 5,6-dihydroxyindole rather than to 5,6-dihydroxyindole-2-carboxylic acid. An interesting issue which emerged from recent studies was the modulation of ink production and ejection by the *N*-methyl-D-aspartate (NMDA)-nitric oxide (NO)-cyclic GMP (cGMP) signalling pathway. Experimental evidence was presented that stimulation of NMDA receptors causes a marked elevation of cGMP levels, activation of tyrosinase and increase of melanin synthesis in the mature portion of the gland, via the NO-guanylyl cyclase interaction. The possible functional significance of ink production and ejection by Sepia was addressed in the following discussion. In the light of a very recent work demonstrating that tyrosinase was the factor responsible for the marked cytotoxic effects of Sepia ink new interpretations of the defence mechanism of ink ejection were offered. The properties and functional significance of Sepia ink melanin were also the focus of a poster communication presented by the group of dr. J.D. Simon from Duke University in Durham. In this, the ability of Sepia melanin to act as metal ion exchanger was reported while the displacement of Ca and Mg by heavy metals adsorbed was taken as consistent with the role of melanin in the regulation of Ca homeostasis recently proposed in the literature .

Plenary Symposium V: Hair and Pigmentation

Chairs: D. Van Neste, J. De Weert

Contributed by J De Weert (Ghent)

The first speaker of the session was Dr. Tobin from the department of Biomedical Sciences of the Bradford University (England). He presented the results of his research on the action of β-endorphin as mediator of human skin pigmentation especially in human hair follicle. A functional role for β-endorphin was assessed in hair follicle melanocyte cultures by direct stimulation with the peptide. He demonstrated the expression of the receptor in cultured cells and also *in situ*. The receptor is present in the melanogenic bulbar cells during the anagen phase. Functional studies showed that β-endorphin has potent melanogenetic, mitogenic and dendritogenic effects so that it can be stated that the peptide plays an important role in the regulation of human hair pigmentation.

The second lecture, presented by Dr. S. Como (L'Oréal Recherche, France) was an overview of the current knowledge about the events that cause and control natural greying of the hair with age. Although the exact mechanism of hair whitening is still unclear, the hypothesis of a gradual and specific melanocyte depletion that affects not only the bulb melanocytes but also the pool of quiescent melanocytes in the outer root sheath is generally accepted.

On behalf of Dr. V.A. Botchkarev, Prof. Barbara Gilchrest (Boston University School of Medicine - USA) proposed the results of an original study concerning the molecular control of the cyclic regeneration of the hair pigmentary unit in C57BL/6 mice. The renewal of the hair pigmentation unit is linked to the anagen stage of the hair cycle. The prominent role of the stem cell factor was demonstrated by the expression of its receptor c-kit by the melanin-producing melanocytes. The blockade of the c-kit receptor induces depigmentation of the hair associated with decrease in melanocyte proliferation and differentiation. In the next hair cycle the previously treated hair follicles show fully pigmentation so that the hypothesis can be accepted that the melanocyte stem cells are not dependent on the stem cell factor/c-kit unit.
The three lectures were excellently documented and presented on a didactic way. A short discussion treated of the role of melanogenesis in the hair growth cycle.

Plenary Symposium VI: Pigmentary genes

Chairs: R. Spritz, C. Goding, F. Beermann

Contributed by F. Beermann (Lausanne)

The session on pigmentary genes was opened by a special lecture of Dorothy Bennett (St. George`s Hospital Medical School, London, UK), who reviewed the current status of coat color genes, and reported on establishing cell lines from mutant mice. So far, at least 127 different gene loci have been identified in the mouse, of which 63 have been cloned and characterized in more detail. Most of these affect melanocyte development and components of the melanosome, and a detailed listing can be found in a recent review article (Bennett and Lamoreux, Pigment Cell Research 2003;16, 333-344). Many of these mutations will now be used to establish cell lines, by breeding onto a p16 (p16INK4A, p19ARF)-deficient background to prevent senescence. For every project - and so far 18 are finished - 3 independent lines are established. At the end of the lecture, an interesting outcome of one of the mutant lines was reported. One of these established lines, which originates from a Microphthalmia allele (*Mitf*^{vit}), showed a revertant phenotype. Whereas 2 lines were rather unpigmented and slowly growing, the third line was variant, larger in cell size and showed increased pigmentation. It will now be a subject of further analyses to reveal, whether this is due to a compensatory mutation elsewhere in the genome, or of a remutation at the *Mitf* gene locus itself.
Eugene Healy (University of Southampton, UK) addressed the increased susceptibility of mice and humans which are mutant/variant at the MC1R (melanocortin receptor), by using transfections of mutant and wildtype MC1R into mouse and human melanoma cell lines. The results show that αMSH augments UV-R induced cell death only in wildtype MC1R transfected cell lines, without any effect on DNA damage or repair. Future studies which might implicate transgenic mice, should reveal whether individuals with wildtype or mutant MC1R are differently susceptible to UV-R induced melanoma formation.
Jan Tavernier (Ghent University, Gent, Belgium) presented a new general method, useful in studying specific protein-protein interactions. The system is called MAPPIT (Mammalian Protein-Protein Interaction Trap) and is based on cytokine receptors and ligand-dependent STAT activation (Eyckerman et al. 2001, Nature Cell Biology 3, 1114-1119, Lemmens et al., 2003, Nucleic Acids Research 31, e75). Following these 3 invited lectures, 2 short selected papers were presented.
Friedrich Beermann (ISREC, Epalinges, Switzerland) had generated knock-out mice lacking Dopachrome tautomerase (*Dct*$^{tm1(Cre)Bee}$), and these mice are completely viable, showing only coat color phenotype and problems in growth of primary melanocytes.
P. Crepaldi (Universita di Milano, Milano, Italy) searched for ways of uniquely identifying cattle breeds in Italy and reported on polymorphisms in melanocortin 1 receptor (MC1R). Using 213 animals from 9 different breeds, 6 polymorphisms were observed. It remains to be proven, whether these polymorphisms are useful in identifying the breed, and to distinguish it faithfully from other breeds.

Plenary Symposium VII: Melanoma and nevi: the genetics

Chairs: A. Taïeb, S. Pavel, D. Bennett

Contributed by A. Taïeb (Bordeaux)

The 2002 Nature paper on the high frequency of single point mutation in exon 15 of the B-Raf gene in melanoma (Nature. 2002 ;417:949-54.) has arisen much hope in the fields of diagnostic, prognostic markers and most of all therapy of this dreadful cutaneous neoplasm. So far, predisposing mutations were found in a minority of familial cases. Another recent report (Hum Mutat. 2003;21:327-30) did not find the B-Raf exon 15 mutation in germline DNA of familial cases suggesting that it is a somatic mutation only associated with melanoma development and/or progression. The session was and occasion to review this new field and its recent advances.
C Wellbrok, Institute of Cancer Research, London indicated that to date, over 30 mutants have been identified, most of them in two clusters in the kinase domain. However, one mutation, a glutamic acid for valine mutation at position 599 is the most frequent mutant, accounting for over 80% of the mutants that have been identified. However, the specificity of Braf mutations is low. BRAF is mutated in a variety of human cancers. Functionally, most mutant proteins can stimulate the activation of endogenous ERK in mammalian cells. In melanocytes ERK could act on the degradation of MITF. In culture, Braf mutants induce both a loss in pigmentation and dendricity.
A.S. Yazdi, Dermatology, University of Munich, reported a study on the major exon 15 T1796A BRAF mutation in primary malignant melanomas including melanoma in situ. The frequency of the specific T1796A mutation in primary malignant melanoma was, compared to that reported in melanoma cell lines, quite low 29% (28/97). Melanoma metastases showed a similar rate (21%). In non cancerous lesions, the mutation was also found. Benign compound acquired nevi had

the highest frequency of mutations (74%). However, in both Spitz nevi and blue nevi, mutations were not detected, suggesting another pathway for tumour genesis.
This study indicates, as other recent reports, that there was a premature hype on a possible new major melanoma gene. BRAF and N-RAS mutations are rarely both present in the same cancers but the cancer types with BRAF mutations are similar to those with RAS mutations. The inappropriate regulation of the downstream ERKs (the p42/p44 MAP kinases) is possibly a major contributing factor in the development of these cancers.

Plenary Symposium VIII: New technical approaches in pigment cell research

Chairs: V. Hearing, C. Goding

Contributed by C. Goding (The Chart Oxted)

In the session entitled "New technical approaches in pigment cell research" Amir Yazdi (Munich) talked first about the application of laser capture microdissection. This technique enables a fraction of cells within a heterogenous population to be examined, for example melanoma cells within a naevus that would contain a mixture of normal melanocytes, melanoma cells in various stages of transformation, as well as keratinocytes. The technique can be applied to cryosections as well as paraffin embedded sections providing the cells of interest can be distinguished for example by morphology, immunostaining or GFP expression. The region of interest within a section is overlaid with a thin thermosensitive plastic film and a laser is used to melt the plastic film onto target cells identified by microscopy, enabling them to be removed from the cell population by raising the film from the surface of the target material. Subsequent downstream analysis can them be performed including gene expression analysis or the identification of gene specific mutations. The great advantage of the technique is that it can be used to provide a high degree of enrichment of a cell type that may represent just a small fraction of the target material and can also enable mutations of gene expression profiles of different cells in the same population to be compared. The major disadvantage is the high cost of the equipment needed.
Dr Yazdi used the system to examine the frequency of B-Raf mutations in primary tumour material with the surprising conclusion that there appeared to be little corellation between activating B-Raf mutations and the presence of melanoma cells within individual naevi. For example, some apparently normal cells within naevi had mutant B-Raf while the apparent tumour cells did not. However, it is possible that assessment of neavi based on morphology is not sufficient and that better markers that distinguish between normal melanocytes and melanoma cells are needed.
Katarina Wolf (Würzburg) spoke on the subject of melanoma migration and invasion using an three-dimensional collagen matrix model in vitro and advanced microscopy techniques. She presented real time movies showing the use of integrins and matrix-degrading proteases in the migration process of single cells or tumor cell groups (clusters) as they move through the matrix. First, a combination of methods was used to visualize pericellular protease function leading to matrix breakdown (e.g. in situ degradation of quenched FITC-collagen; an antibody recognizing degraded collagen; dynamic confocal reflection imaging). These techniques show matrix cleavage at growing pseudopods and along the cell body when the cell is constricted and compressed by fibers. When proteases were blocked by a cocktail of protease inhibitors, mesenchymal moving cells continued to move by conversion to an amoeboid style of movement, designated mesenchymal-amoeboid transition (MAT) 1. Similarly, after blocking of ß1 integrin function in single cells as well as cell groups using various strategies, the conversion to an amoeboid mechanism of movement was obtained. She concluded, that amoeboid movement might represent a novel mechanism of melanoma cell migration to rescue the dissemination process despite pharmacological targeting of proteases and integrins, implicating an unexpected degree of plasiticity in cancer cell migration.
Lluis Montoliu (Madrid) described the analysis of the tyrosinase locus control region (LCR), a region of DNA lying some 15 kb upstream from the promoter. LCRs are complex higher order chromatin structures that include several elements required for the correct spatio-temporal expression of a gene and which act to protect a particular locus from the effects of elements controlling expression of neighbouring genes. The tyrosinase LCR spans around 2.1kb and co-localises with a DNAse1 hypersensitive site that indicates a region of more open chromatin. Transgenic mice expressing the *tyrosinase* proximal promoter are highly subject to position effects resulting in the transgene being stringly influenced by the chromatin in surrounding the site of integration in the genome. This variation is overcome by the LCR, resulting in greatly enhanced tyrosinase expression. The tyrosinase LCR could also insulate *Drosophila white* minigene from chromosomal position effects and displayed properties associated with boundary elements. In mice it appears that the tyrosinase locus Line1 element lies immediately upstream from the LCR and it seems likely that one role of the LCR is to prevent a repressive chromatin conformation incompatible with gene expression spreading from the line1 element towards the promoter. Although further work is necessary to identify the factors contributing to LCR function, the tyrosinase LCR represents a good model for examining the role of LCRs to regulate gene expression through control of chromatin dynamics.
Ludovine Petit (Liége) examined the subclinical mottled pigmentation on sun-exposed areas in subjects older than 60 years by means of a Visioscan. This video camera equipped with an internal UV light emitting unit was directly applied onto the skin to disclose or enhance the contrast between the hyperpigmented areas and the surrounding skin. Image analysis revealed three main patterns of mottled melanoderma: the speckled perifollicular type, the streaky type and the accretive interfollicular type. There was a great inter-individual variability but the intra-individual differences were most often minimal. Moreover, dermal aging was assessed with a Densi Score device. It was not correlated with the extent of the darker spots of melanoderma. In her study, age influenced dermal aging but had no influence on mottled melanoderma, suggesting that UV light affects the melanocytes earlier in life while dermal aging and photoaging continue to progress during lifetime and particularly after 60 years of age.'

In the last talk of the session Veronique Delmas (Orsay) talked about generating mice expressing the CRE recombinase from the mouse tyrosinase promoter/enhancer, the aim being to generate melanocyte-specific gene knockouts. The mice express CRE in the correct spatio-temporal fashion and were used to make melanocyte-specific knockouts of both the insulin-like growth factor receptor gene (Igf1r) and for β-catenin. The use of this system highlights the beauty of melanocyte development as a model for studying gene function: non-conditional Igf1r and β-catenin knockouts are postnatal and embryonic lethal, respectively, whereas in contrast the melanocyte-specific knockout for Igf1r had no phenotype while that of β-catenin yielded white mice. It is possible, however, that a subtle coat color phenotype could not be easily observed for the Igf1r melanocyte-specific knockout as these mice had a mixed 129/Sv, DBA/2, C57BL/6 genetic background. Nevertheless, the availability of the tyrosinase CRE mice will enable the melanocyte system applied to the functional analysis of the enormous number of genes whose disruption leads to an embryonic lethal phenotype.

Plenary Symposium IX: Experimental treatments in melanoma

Chairs: M. Picardo, L. Brochez

Contributed by M. Picardo (Rome)

The plenary symposium IX held on September 18 afternoon hosted one invited lecture delivered by Ghanem Ghanem (Brussels) and a free communication held by V. Horak (Prague).

The subject of Ghanem's talk was the mechanism of anticancer targeting of PSF, a prodrug containing *m*-sarcolysin, analogue to melphalan. The first results concerned the metabolisation of PSF, delivered by cyclodextrins, in plasma and in white and red blood cells. Erythrocytes are involved in the metabolism of PSF into *m*-sarcolysin. From the kinetic study it resulted that PSF binds quickly to red blood cells (10 min) and one third is transformed into *m*-sarcolysin. In order to investigate the implication of enzymatic catalysis in the process, red blood cells were treated with EDTA (to inhibit metalloproteases) and exposed to PSF, and it was concluded that PSF is degraded by proteolytic anzymes, including metalloproteinases. Melanoma cells were also able to convert PSF into *m*-sarcolysin but at a lesser extent than erythrocytes. Moreover, cell metabolisation of PSF showed the formation of three different drug metabolites, including *p*-fluorophenylalanine. The activation of MMP-2 and MMP-9 was induced by pro-inflammatory cytokines frequently found in tumors. The cytotoxicity of PSF and *m*-sarcolysin on red blood cells and melanoma cells was then evaluated at 24 and 48 hrs.

In his communication, V. Horak, from the Institute of Animal Physiology and Genetics in Prague, presented a study regarding the induction of T-cell mediated anti-tumor immunity in MeLiM miniature pigs, developed as animal model for human melanoma. Sixty percent of these darkly pigmented animals present hereditary melanoma with histologic and biochemical similarities with human melanoma. This model is presented as useful for the development of antitumor therapies. Melanoma in such animals, manifests with multiple skin metastasis. Devitalisation of skin tumor as therapeutic approach to melanoma was first advanced by Karel Kortyn. In this study, lymphonodes were excised before and after 1 month from devitalisation, as well as after 6 months following the complete destruction of the tumor. Melanoma cells were destroyed in lymphonodes, and the expression of fibronectin was abolished in devitalised tumors after two months. From these results it was argued that devitalisation of tumor induces a cell-mediated immune reaction. The expression of heat shock proteins (HSP) was induced during devitalisation, in particular the expression of HPS70 and gp96 were abolished after 2 monts of devitalisation. The HPS expression was assessed by immunohistochemistry. Moreover, the leucocytes subsets were assessed in the pigs. The immunophenotyping of lymphocytes showed an increase of $CD4^+$ positive cells after devitalisation. Whereas, immunoglobulins level didn't change before and after devitalisation. To summarise, the devitalisation effects were of a decreased expression of extracellular matrix proteins. The level of HSP was increased and a higher infiltration of cytotoxic and helper T-lymphocytes was detected.

Plenary Symposium X: Biogenesis and movement of melanosomes and related organelles-

Chairs: J. C. García-Borrón, J.L.W. Lambert

Contributed by W Westbroek (Ghent)

Original findings on molecules that play a key role in movement and/or biogenesis of melanosomes were presented. Cultured melanocytes and fibroblasts derived from patients or mouse models suffering from rare disorders like Hermansky Pudlak syndrome and Griscelli syndrome known to affect biogenesis and transport of lysosomes and related organelles, were frequently used to study the function of proteins in these processes. Jordens and co-workers found that rab7, a member of the ras-like small GTPases and regulators of vesicle movement, sorting and secretion, interacted with lysosomal compartments. Active rab7 recruits RILP (Rab7 Interacting Lysosomal Protein) via a direct interaction while RILP recruits the minus-end directed dynein-dynactin motor complex to regulate lysosomal transport. New experimental data on primary human melanocytes revealed partial colocalization between rab7 and melanosomes and also rab27a, a small GTPase known to interact with the melanosome membrane where it acts as a receptor for melanophilin, a rab27a effector, and myosin Va, an actin dependent motor protein. Overexpression of RILP gave partial perinuclear clustering of melanosomes and rab27a. Dominant negative rab27a induced clustering of melanosomes in the perinuclear region; this could be abolished by co-transfection with dominant negative rab7, dominant negative RILP and p50, which is known to disrupt dynein-dynactin motor function when over-expressed. Jordens and co-workers suggested that rab7 and rab27a could regulate each other. This would provide the first study on the interrelationship between two different rab proteins. Further experiments on melanocytes derived from patients with Griscelli syndrome type II (rab27a protein deficient) need to confirm their hypothesis. In addition to the above discussed dynein dependent transport in human cells, the data of Aspengren and co-workers stated that spectrin, a membrane-associated dimeric protein that forms a complex with ankyrin and actin, plays a

role in dynein-dependent movement of melanosomes in frog melanophores. Immunocitochemistry, co-immunoprecipitation and immuno-electron microscopy revealed that dynein and spectrin could be found in close proximity on the melanosomal membrane. This is the first time that a putative role for spectrin is described in melanosome transport. Raposo and coworkers presented a journey through melanosome biogenesis by regular and immuno-electron microscopy obtained data. Using endocytotic and lysosomal tracers, it was clearly stated that, although melanosomes share features to lysosomes, planar clathrin-coated endocytic structures play a role in premelanosome formation and that mature melanosomes are different from lysosomes. Cleavage of Pmel17, which routes to stage II premelanosomes, by proprotein convertases initiates fibril formation in maturating melanosomes. Tyrp1 is present on stage III and IV melanosomes via routing through TGN-coated vesicles. This overview gave an insight into the mechanisms implicated in protein transport routes to melanosomes and provided a basis for understanding the malfunctions in Hermansky Pudlak syndrome discussed by Huizing and coworkers and Sviderskaya and coworkers. Huizing and coworkers gave a clear overview on the six human forms of Hermansky-Pudlak, a disorder characterized by abnormal biogenesis of lysosomes, melanosomes and platelet dense granules. The biochemical function of HPS1, HPS3, HPS4, HPS5 and HPS6 is still not elucidated. HPS1 and HPS4 as also HPS5 and HPS6 interact with each other and are part of different BLOCs (Biogenesis of Lysosome-related Organelles Complex). No interaction partners are identified yet for human HPS3. From transfection experiments on HPS3 fibroblasts with CD63 and LAMP-1, both lysosomal markers, it was carefully suggested that clathrin could be a potential HPS3 binding partner. HPS2 seems to be the only form that is biochemically characterized. Huizing and coworkers found that malfunctioning of the ß3 subunit of the adaptor complex AP-3 causes misrouting of tyrosinase to melanosomes in melanocytes derived from HPS2 patients. Raposo and coworkers are currently studying the role of AP-3 in trafficking steps of melanogenic enzymes from the TGN and early sorting endosomes to maturing melanosomes in melanocytes by means of electron microscopy. Here, HPS2 melanocytes could also form the ideal study material. Sviderskaya and coworkers described altered tyrosinase localization in four different mouse models of Hermansky- Pudlak by means of DOPA staining and electron microscopy.

It was already demonstrated that glycolipid synthesis was necessary for melanin production in melanosomes. It was shown that in a glycolipid negative melanoma cell line, tyrosinase was not targeted to melanosomes but accumulated in the Golgi complex. Smit and coworkers performed lipid content measurements in melanoma and melanocyte cultures with low and high degree of pigmentation. A higher ratio of glucosyl ceramide/ceramide was found in more melanized cell cultures. Treatment of melanocytes with an iminosugar homologue, known to inhibit glycolipid synthesis, resulted in a strong reduction of melanin production and reduced tyrosinase activity in melanocytes of both light and dark skin type origin and this in a dose dependent manner. They suggested that, next to the already existing commercial depigmenting agents acting on melanogenic enzymes or on the PAR-2 receptor, expressed on keratinocytes and hereby preventing intracellular transfer of melanosomes from melanocytes to keratinocytes, regulation of glycolipid production by an iminosugar homologue could offer an alternative to influence pigmentation in human melanocytes. Suggestions indicated that it would be of great importance to additionally test the iminosugar homologue and its potential toxicity also on keratinocytes and fibroblasts, cell types in close proximity to melanocytes *in vivo*.

Plenary Symposium XII: UV and pigmentation

Chairs: P. Verrando, M. Garmyn

Contributed by P. Verrando (Marseille)

Markus Böhm's invited lecture dealt with the *in vivo* effects of UV on the skin melanocortin signal pathway, *in situ* induction of interleukin 10 (IL-10) and some subsequent effect with regard to apoptosis in melanocytes. After UV irradiation by a solar irradiator of human skin from volunteers, cutaneous suction blisters were realized and analyzed at the transcriptional and protein levels for pro-opiomelanocortin (POMC), the precursor of α-Melanocyte Stimulating Hormone (α-MSH), α-MSH itself, the melanocortin-1 receptor (MC1R), as well IL-10 (Schiller *et al.* In revision).

POMC, MC1R and IL-10 mRNA increased in the suction blister roofs from the skin irradiated by UV as compared to non-irradiated ones. An increase in the release of α-MSH and IL-10 was also noticed (ELISA) in the fluids of the suction blister roofs from UV-irradiated skin. This effect could be abolished by a broad-spectrum sunscreen. Therefore, an inductive effect of UV on the POMC signal pathway was demonstrated *in vivo* by this technique.

Further experiments were carried out on cultured normal human melanocytes (NHM) to assess the recently suggested protective effect of α-MSH on UV-induced apoptosis in these cells. After a 72 h deprivation of bovine pituitary extract required to culture NHM, cells were subjected to UVB alone (60 mJ/cm^2), or a combination of UVB and 6 h later, α-MSH (10^{-6} M). Cell death was monitored after 18 h, and results were also confirmed by the status of annexin V (cytofluorimetry). The results demonstrated that α-MSH alone did not affect cell viability, while it protected the cells up to 50 % from the deleterious effect of the UVB radiation. The protective effect of α-MSH against UVB-induced apoptosis in NHM was associated with the reduced formation of pyrimidine dimers being the predominant DNA lesion induced by UVB (yet occurring at 15 mJ/cm^2). This protective effect did not involve the status of the apoptotic protein effectors Bcl-2, Bcl-x or Fas, nor modifications of the cell cycle progression.

In addition, in cultured dermal fibroblasts which express MC1R, α-MSH is able to modulate the collagen synthesis activated by TGFß, but do not suppress induced levels of collagen transcripts. *In vivo*, microscopic examination of elastic fibers in mouse skin sections showed an anti-fibrogenic activity of the hormone.

As concluded by the speaker, melanocortins induced in skin cells by UV may have a role in immunomodulation, survival and integrity of the skin in addition to that of acting on pigmentation

Plenary Symposium XIII: Clinical aspects of melanoma

Chairs: J.M. Naeyaert, C. Cocquyt

Contributed by Lieve Brochez

Plenary Session XIII started with an invited lecture entitled "Current adjuvant therapy in melanoma", by L Brochez (Ghent). Adjuvant therapy is aimed at reducing the risk of relapse in patients at high risk of disease recurrence who are free of macroscopic disease at the time the treatment is started. Several agents such as non-specific immunotherapy (BCG,…), chemotherapy (DTIC,…), retinoids, hormonal substances, radiotherapy,… have not proven to be of benefit in the adjuvant treatment of melanoma. More recently attention has focused on specific immunotherapy (induction of tumour-targeted immune response by means of vaccination) and interferon. The presentation focused on interferon (IFN) in the adjuvant setting, because of its availability and controversy. Three randomized trials (ECOG 1684, ECOG 1690, ECOG 1694) investigated the use of a high dose IFNα-2b regimen in patients with thick primary tumours (Breslow > 4 mm) or regional lymph node metastasis. ECOG 1684 and ECOG 1690 compared high dose IFN to observation, ECOG 1694 compared high dose IFN to a ganglioside GM2/KLH/QS21 vaccination arm. All studies seem to confirm a positive effect on disease-free survival, which is also confirmed in a long-term follow-up of the patients in the ECOG 1684 trial. The effects on overall survival are less uniform (positive in ECOG 1684 and 1694, negative in 1690), and different explanations and interpretations of these results are given by proponents and opponents of the high-dose IFN treatment. Treatment with high dose IFN is accompanied by a considerable toxicity and cost, and there are no updated cost-effectiveness analyses.

Studies with low-dose IFN in the adjuvant setting seem to confirm an effect on delaying disease relapse. This effect seems to be lost quickly after the therapy is discontinued, which triggers the question how long the therapy should be continued. There seems to be no benefit on overall survival. In conclusion, IFN seems to be able to delay disease recurrence. There are still uncertainties about a positive effect on overall survival of high dose IFN. The results of ongoing clinical trials with an intravenous one month treatment with high dose IFN (ECOG 1697, Sunbelt Melanoma Trial), with intermediary dose regimens (EORTC 18952) and with pegylated interferon (EORTC 18991) are awaited. In the meantime the current available medical evidence can be discussed with the patient, who should be involved in the decision process.

The second invited lecture of the session , "Sentinel node biopsy as standard of care in melanoma ?" was delivered by B. B. R. Kroon. At present the technique of sentinel node biopsy is mature, with experience of more than 10 years. The presentation discussed the pros and cons of this surgical procedure. Possible advantages of this surgical procedure are 1) its strong prognostic significance (5-year overall survival decreases from 88% in sentinel node negative patients to 63% in sentinel node positive patients ($p<0.001$)), 2) improved staging of patients with identification of unexpected drainage, and 3) a possible small survival benefit, although the latter needs to be confirmed.

The disadvantages of the procedure relate to the additional surgery requiring general anesthesia, prolonged operation time and extra costs. Failure of the technique to identify regional micro-metastasis is estimated at 7 to 27% and attributed to unreliability of lymphoscintigraphy to identify the sentinel node (reproducibility of 88%), failure of pathology to detect micro-metastasis and bypass of the sentinel node by the tumour cells. Another disadvantage is the possibility of surgery-related comorbidity such as lymph edema and lymphocoele. In addition an increased risk of in-transit metastasis in node-positive patients is feared: the incidence of in-transit metastasis after positive sentinel node biopsy is estimated at 19 to 27% (MD Anderson, the Netherlands), compared to 8% of the patients with palpable lymph nodes and delayed lymph node dissection.

In conclusion, the main advantage of the technique at this point is its strong prognostical significance. However it is an invasive staging technique and for the moment there is no standard adjuvant therapy in patients diagnosed with lymph node metastasis. A possible survival benefit of the early detection and treatment of stage III disease is the subject of current investigation (Melanoma Selective Lymphadenectomy Trial). Subgroup analysis in clinical trials of elective lymph node dissection (prophylactic excision of draining lymph node station) suggested a possible survival benefit in not-ulcerated melanoma on the limbs with Breslow-thickness from 1 to 2 mm, and in trunk melanoma in males with Breslow-thickness from 1.5 to 4mm.

The last invited lecture in the session, by A. Demunter (Leuven), and dealt with "Prognostic factors in melanoma". Efforts to understand the molecular basis of melanoma progression are continuing. Mutations in many different genes have been described in melanoma, including the N-*RAS* genetic alterations with most mutations in codon12 and 13 (exon1) and in codon61 (exon2). These mutated *RAS* proteins are resistant to inactivation by GTPase activating proteins, surmounting in continuous proliferation signals to the nucleus. The exact role of these mutations in the process of tumour progression remains to be elucidated. The presentation discussed the results of *RAS* gene mutation analysis in distinctive tumour progression stages of melanoma (7 nevi, 69 primary melanomas and 35 melanoma metastasis) using cells isolated by laser-assisted microdissection and subjected to DOP-PCR (degenerated oligonucleotide primed polymerase chain reaction). Mutants were detected by subsequent PCR amplification of exons 1 and 2 of the N-*RAS* gene and visualization of the products on denaturing gradient gel electrophoresis. Sequencing was done in case of aberrant migration pattern.Thirty percent of the samples displayed the classical mutations at codon 12 and codon 61. No codon 13 mutations were observed. The mutations occurred already in the early growth phases (mainly codon 12 mutations) and even in some associated nevi, and were preserved throughout tumour progression with the possible acquisition of additional mutations (mainly codon 61 mutations). This suggests clonal relationship between the different melanoma progression phases and even between melanoma and associated nevi. In 10% of the cases, a not previously reported codon 18 mutation in exon 1 of N-*RAS* was detected. This mutation presented in early tumour progression stages (primary melanoma, not metastasis). In a retrospective analysis, melanomas with this mutation were significantly thinner and had better prognosis than in the other patients. There was no significant difference in GDP/GTP affinity of the mutated protein compared to the wild type ras protein.

The session ended with a free communication, “Analysis of APAF-1 expression in human cutaneous melanoma progression”, presented by M.G. Paggi (Rome). Defects in apoptotic pathways are important in cancer progression. APAF-1 plays a role in mitochondria-dependent apoptosis through activation of caspase-9. Western blot analysis demonstrated APAF-1 downregulation in metastatic melanoma cell lines, compared to primary melanoma cell lines. Immunhistochemical analysis demonstrated significant lower staining in melanomas compared to nevi. In addition there was a significant lower expression of APAF-1 in thick (Breslow >0.76mm) compared to thin (Breslow<0.76mm) melanomas, and in melanoma metastasis compared to primary melanomas.

Plenary Symposium XIV: Pigmentary skin disorders

Chairs: B.A. Gilchrest, K. Ongenae

Contributed by Katia Ongenae (Ghent)

The session was opened by DJ Gawkrodger, who spoke on autoimmunity and vitiligo. On the background of known criteria for autoimmune disease, the arguments for considering vitiligo an autoimmune disease were presented. Among them is the observed association of vitiligo with other clinical conditions thought to be autoimmune in origin: for example thyroid disease, pernicious anemia, polyglandular syndromes. Also elevated levels of organ specific antibodies (directed against thyroid gland or gastric parietal cells) have been detected in vitiligo. Repigmentation is obtained in vitiligo with treatments which are known to have an immunosuppressive effect for example topical steroids, PUVA and Cyclosporin A. A significant association of vitiligo with allele HLA-DR4 is reported for several populations. The potential role of cellular and humoral immunity in the pathogenesis of vitiligo is reviewed. On a more experimental level circulating antibodies to pigment cell antigens (enzymes, structural antigens) have been detected. When injected into nude mice grafted with pigmented skin, a vitiligo-like depigmentation is observed in grafted pigmented skin. Infiltrating activated T lymphocytes are observed at the periphery of vitiligo lesions and circulating cytotoxic T lymphocytes directed against melanocyte antigens were observed in vitiligo. These cells are able to destroy melanocytes and are related with the activity of the disease. The Smyth chicken is an animal model of depigmentation were cellular and humoral autoimmune mechanisms are described. These observations support the idea of an autoimmune mechanism in the pathogenesis of vitiligo. It is however not clear if these autoimmune phenomena are of primary or secondary origin.

The next talk, by R. Spritz, dealt with the epidemiology and genetics of generalized vitiligo. Vitiligo is a complex disorder with multiple causative factors, both genetic and non-genetic. Although the specific causative factors are not yet known, the biological basis of vitiligo is likely autoimmune in most or all cases. The pattern of inheritance of vitiligo is indicative of a polygenic, multifactorial condition, with about one-third of patients having an affected first-degree relative and concordance in monozygous twins of 23%. Vitiligo is highly associated with certain other autoimmune diseases--autoimmune thyroid disease, pernicious anemia, Addison's disease, lupus, and adult-onset insulin-dependent (autoimmune) diabetes mellitus, and the increased frequencies of these same disorders in patients' first-degree relatives (regardless of whether or not those persons have vitiligo) indicates that this autoimmune diathesis has a genetic basis. By analysis of 102 families with multiple cases of vitiligo, this group has demonstrated significant genetic linkage of four loci genomewide: AIS1 (chromosome 1p31.3-p32.2), AIS2 (chromosome 7), AIS3 (chromosome 8), and SLEV1 (chromosome 17), none of which correspond to the locations of 'candidate genes' previously suggested for vitiligo. Interestingly, they detected no linkage to the MHC region of chromosome 6. Stratification of the 102 families into two equal groups based on the occurrence or absence of other vitiligo-associated autoimmune diseases shows that the AIS1, AIS2, and SLEV1 linkage signals derive from the 'autoimmunity' families, whereas the AIS3 signal derives from the 'vitiligo-only' families. Thus, different genes, perhaps acting in different combinations, appear to predispose to vitiligo in different types of families. For example, in one large family AIS1 appears to predispose to vitiligo, Hashimoto thyroiditis, and Addison's disease, whereas another gene, on chromosome 6, predisposes to Hashimoto disease; co-inheritance of both genes is necessary and sufficient to specify the occurrence of Hashimoto disease in this family. Nevertheless, it remains to be seen what roles these genes may play in more common, non-familial cases of vitiligo. Efforts towards identification of the AIS1 gene are in progress.

C Dierickx then presented a lecture on the use of lasers in pigmentary disorders. Selective destruction of pigment by laser treatment can be achieved by delivery of high energy at the wavelength absorbed by the target chromophore. The localization of the pigment, the way it is packaged and the nature of the pigment are determining factors in laser treatment of pigmentary disorders. Short-pulsed (nanosecond) lasers have an extremely short pulse duration. They mediate a photoacoustic destruction of melanosomes. Their treatment gives an immediate whitening of the pigmented skin. Millisecond lasers have a longer pulse duration. They mediate a selective heating and destruction of melanocytes. Their treatment can result in a sloughing of the pigmented skin. Various treatment indications are reviewed with their expected treatment results. Freckles, lentigines, nevus of Ota and blue nevus react well to nanosecond lasers. Café au lait maculae, nevus spilus and Becker’s nevus give varying results. Treatment of acquired nevocellular nevi and congenital nevi with short-pulsed lasers often give partial clearance and recurrence. Laser treatment of those nevi is still a controversial issue because of the risk for malignant degeneration. Millisecond lasers can also be used for CALM, Becker’s nevus and congenital nevi. Finally the treatment of localized vitiligo with lesion-directed UV phototherapy (308 nm excimer laser and filtered UV source) seems to give promising results and offers the advantage of fewer treatments and lower cumulative doses. However further controlled studies evaluating this treatment option are needed.

JM Naeyaert reported on the surgical treatment of vitiligo. Surgical treatment of vitiligo is considered when the disease is stable and not responding to medical treatment. The common basic principle of the reviewed surgical techniques is the transfer of autologous pigment cells from a pigmented donor area to a depigmented acceptor area. This can be achieved with tissue grafts (e.g. blister roofs, split thickness grafts, punch grafts) and cellular grafts (cultured and non cultured cell suspensions) each with their advantages and disadvantages. The transplantation technique using autologous non cultured

epidermal cells has been described earlier by this research group: a split thickness graft is taken from pigmented buttock skin, trypsinized and resuspended in low calcium melanocyte medium enriched with hyaluronic acid while the acceptor area is prepared by CO_2 laser abrasion under topical anesthaesia. A double blind placebo controlled study is presented aiming at assessing the efficacy (with digital image analysis) and the safety of this treatment and evaluating the mechanism of repigmentation. 33 symmetrical paired lesions (in 28 patients) were included and treated (left-right) either with a cellular suspension or a placebo suspension. Repigmentation of at least 70% in 55%, 57% and 77% of the patients with stable disease was observed at the treated side after 3, 6 and 12 months respectively. Placebo treated sites were displaying no repigmentation and patients with doubts about the stability of the disease were displaying no repigmentation. The pattern of repigmentation is diffuse. Except for a change of skin texture or transient hyperpigmentation, no adverse side effects were observed. This study presents a safe and easy, one-day procedure for transplantation of leucoderma; proves the role of the transplanted cells in the obtained repigmentation and highlights the importance of stability of the depigmentation disorder in obtaining a successful result.

Y VanderHaeghen discussed the reproducible clinical dermatological imaging with commercially available digital cameras. Digital imaging in dermatology encounters similar problems as traditional photography. Changes in exposure, white balance and lighting hazards the acquisition of reproducible images of the same object over different time points. Transforming the image data to a defined standard color space enables the improvement of the reproducibility of skin images taken with commercially available digital cameras. A card with 12 color patches with known colorimetric properties is included in the image. Analyzing the properties of these patches in the acquired image determines a mathematical transformation to the standard color space applicable to the whole image. This method has been evaluated for its reproducibility and accuracy. It enables proper visual comparison between images and exchange of images as well as quantitative and colorimetric comparison between image regions.

FR. De Gruijl examined the relationship of UV carcinogenesis and melanocytes. UV is a known carcinogenic agent. Point mutations in the P53 tumor suppressor gene offer direct evidence of the link between squamous cell carcinoma and solar UV. Pigmented skin is more resistant to the development of UV induced skin cancer but the exact role of melanocytes is not clear. BRAF (downstream of RAS) was found to be mutated in a majority of nevi and melanoma. Although the dominant point mutation (T1796A) is not characteristic of UV radiation this does not exclude a potential role of UV. Transgenic mouse models may help to clear out how UV radiation impacts on signalling pathways of melanogenesis. It was demonstrated that UV radiation was more targeted at the p16-INK4a/Rb tumor suppressor route than at an RTK-RAS oncogenic route.

The last talk of the session, entitled “The Par-2 pathway is differentially expressed in skin of color”, was delivered by. M Seiberg. Humans are divided into three racial groups: Caucasoid, Negroid and Mongoloid, each associated with a certain skin color. Skin color results from the production and distribution of melanin in the epidermis. This group has demonstrated earlier that the protease-activated receptor-2 (PAR-2), which is expressed on keratinocytes but not on melanocytes, is involved in melanosome uptake via phagocytosis. Modulation of PAR-2 activation with serine protease inhibitors or with PAR-2 activating peptides affects skin color, as shown in human skin grafted on SCID mice. The pattern of melanosome distribution within the epidermis is skin color-dependent. *In vitro*, this distribution pattern is regulated by the ethnic origin of the keratinocytes, not the melanocytes. Therefore, it is hypothesized that PAR-2 may play a role in the modulation of pigmentation in a skin-type dependent manner. This group examined the expression of PAR-2 and its natural activator, trypsin, in human skins with different pigmentary levels. They showed that PAR-2 and trypsin are expressed in higher levels, and are differentially localized within the epidermis, in highly pigmented, relative to lightly pigmented skins. Moreover, highly pigmented skins exhibit a significant increase in PAR-2-specific protease cleavage (activation) ability. PAR-2-induced phagocytosis was found to increase in keratinocytes from highly pigmented skins, suggesting a more efficient melanosome transfer into dark keratinocytes. UV-induced PAR-2 activation *in vitro* followed the same pattern. These results demonstrate that PAR-2 expression and activation correlate with skin color, and suggest the involvement of PAR-2 in pigmentation modulation in different skin types.

CURRENT LITERATURE

1. Chemistry of Melanin and other Pigments

(Dr A. Napolitano)

About forty papers have appeared in the last year dealing with melanin pigments. They report very different aspects of melanins and I would therefore propose a further classification according to the items below.
Numerous works focus on the binding properties of melanin to different drugs. The effects of acid/alkaline extraction procedures on the structure of natural melanins were investigated by Liu et al by a combined approach using microscopy and chemical analysis. The results provide substantial support to the common notion stemming from previous literature data that harsh treatments result in a significant structural alteration. A number of techniques were applied to the investigation of melanin properties, including the variation in size and morphology of synthetic pigments subjected to chemical bleaching (small-angle X-ray scattering, Littrell *et al*), the photoreactivity of eu/pheomelanins and related pigments such as deoxytrichochrome C (ultrafast absorption spectroscopy, Ye, Simon and Saran, Ye and Simon, Ye *et al*). The functions and modifications of retinal epithelium melanins with aging (Sarna *et al*, Dayhaw-Barker P.) were also addressed.
The methods of eumelanin and pheomelanin analysis in tissues by chemical degradation were reviewed (Ito and Wakamatsu) and extended to determination of pheomelanin-related metabolites in urine of melanoma patients (Takasaki *et al*).
Of particular interest is a review reporting the results of extensive studies on the early stages of melanogenesis (Land, Ramsden and Riley). In this, the mechanisms of tyrosinase autoactivation and the chemistry of *ortho* quinone amines are specifically addressed. Experimental data obtained on structurally modified dopaquinones by a combination of enzyme oximetry, pulse radiolysis and chemical oxidation provide firm evidence of nonenzymatic catechol formation during tyrosinase oxidation of phenols. Some of these data are presented in a related paper (Land *et al*, Pigment Cell Res) The chemistry of *ortho* quinones and quinonemethanes related to melanogenesis is described in a paper by Land *et al*
Finally several papers deal with the formation and properties of other melanin-related pigments such as those produced by pathogenic fungi.

MELANIN REACTIVITY AND PROPERTIES:

- Buszman E, Rozanska R.
  Interaction of quinidine, disopyramide and metoprolol with melanin in vitro in relation to drug-induced ocular toxicity. Pharmazie. 58(7):507-11, 2003.

- Chakraborty DP, Roy S.
  Chemical and biological aspects of melanin. Alkaloids Chem Biol. 60:345-91, 2003.

- Dayhaw-Barker P.
  Retinal pigment epithelium melanin and ocular toxicity. Int J Toxicol. 21(6):451-4, 2002.

- Hoogduijn MJ, Smit NP, van der Laarse A, van Nieuwpoort AF, Wood JM, Thody AJ.
  Melanin has a role in Ca2+ homeostasis in human melanocytes. Pigment Cell Res. 16(2):127-32, 2003.

- Koeberle MJ, Hughes PM, Skellern GG, Wilson CG.
  Binding of memantine to melanin: influence of type of melanin and characteristics. Pharm Res. 20(10):1702-9, 2003.

- Koeberle MJ, Hughes PM, Wilson CG, Skellern GG.
  Development of a liquid chromatography-mass spectrometric method for measuring the binding of memantine to different melanins. J Chromatogr B Analyt Technol Biomed Life Sci. 25;787(2):313-22, 2003.

- Littrell KC, Gallas JM, Zajac GW, Thiyagarajan P.
  Structural studies of bleached melanin by synchrotron small-angle X-ray scattering.Photochem Photobiol. 77(2):115-20, 2003.

- Liu Y, Kempf VR, Nofsinger JB, Weinert EE, Rudnicki M, Wakamatsu K, Ito S, Simon JD.

- **Comparison of the structural and physical properties of human hair eumelanin following enzymatic or acid/base extraction**. Pigment Cell Res. 16(4):355-65, 2003.

- Liu Y, Simon JD.
  The effect of preparation procedures on the morphology of melanin from the ink sac of Sepia officinalis. Pigment Cell Res. 16(1):72-80, 2003.

- Ono C, Tanaka M.
  Binding characteristics of fluoroquinolones to synthetic levodopa melanin J Pharm Pharmacol. 55(8):1127-33, 2003.

- Prem P, Dube KJ, Madison SA, Bartolone J.
  New insights into the physicochemical effects of ammonia/peroxide bleaching of hair and Sepia melanins. J Cosmet Sci. 54(4):395-409, 2003.

- Sarna T, Burke JM, Korytowski W, Rozanowska M, Skumatz CM, Zareba A, Zareba M.
  Loss of melanin from human RPE with aging: possible role of melanin photooxidation. Exp Eye Res. 76(1):89-98, 2003.

- Satyamoorthy K, Li G, Van Belle PA, Elder DE, Herlyn M.
  A versatile method for the removal of melanin from ribonucleic acids in melanocytic cells. Melanoma Res. 12(5):449-52, 2002.

- Svensson SP, Lindgren S, Powell W, Green H.
  Melanin inhibits cytotoxic effects of doxorubicin and daunorubicin in MOLT 4 cells. Pigment Cell Res. 16(4):351-4, 2003.

- Wrzesniok D, Buszman E, Karna E, Nawrat P, Palka J
  Melanin potentiates gentamicin-induced inhibition of collagen biosynthesis in human skin fibroblasts. Eur J Pharmacol.;446(1-3):7-13, 2002.

- Ye T, Simon JD, Sarna T.
  Ultrafast energy transfer from bound tetra(4-N,N,N,N-trimethylanilinium)porphyrin to synthetic dopa and cysteinyldopa melanins. Photochem Photobiol. 77(1):1-4, 2003.

MELANIN BIOSYNTHESIS

- Di J, Bi S.
  Aluminum ions accelerated the oxidative stress of copper-mediated melanin formation. Spectrochim Acta A Mol Biomol Spectrosc. 59(13):3075-83, 2003.

- Di J, Bi S.
  Effect of aluminum (III) on the conversion of dopachrome in the melanin synthesis pathway. Spectrochim Acta A Mol Biomol Spectrosc. 59(8):1689-96, 2003.

- Ito S;
  The IFPCS presidential lecture: a chemist's view of melanogenesis. Pigment Cell Res. 16(3):230-6, 2003.

- Land EJ, Ramsden CA, Riley PA.
  Tyrosinase autoactivation and the chemistry of ortho-quinone amines. Acc Chem Res. 36(5):300-8, 2003.

- Land EJ, Ramsden CA, Riley PA, Yoganathan G.
  4-Cyanomethyl-*ortho* quinone tautomerism and the structure of the dienophile in Gates'morphine synthesis. Tetrahedron 59(48):9547-54, 2003.

- Land EJ, Ramsden CA, Riley PA, Yoganathan G.
  Mechanistic studies of catechol generation from secondary quinone amines relevant to indole formation and tyrosinase activation. Pigment Cell Res. 16(4):397-06, 2003.

- Palumbo A.
  Melanogenesis in the ink gland of Sepia officinalis Pigment Cell Res. 16(5):517-22, 2003.

MELANIN ANALYSIS

- Ito S, Wakamatsu K.
  Quantitative analysis of eumelanin and pheomelanin in humans, mice, and other animals: a comparative review. Pigment Cell Res. 16(5):523-31, 2003.

- Parsad D, Wakamatsu K, Kanwar AJ, Kumar B, Ito S.
  Eumelanin and phaeomelanin contents of depigmented and repigmented skin in vitiligo patients. Br J Dermatol. 149(3):624-6, 2003.

- Takasaki A, Nezirevic D, Arstrand K, Wakamatsu K, Ito S, Kagedal B.
  HPLC analysis of pheomelanin degradation products in human urine. Pigment Cell Res. 16(5):480-6, 2003.

PHEOMELANINS

- Di Donato P., Napolitano A.
  1,4-benzothiazines as key intermediates in the biosynthesis of red hair pigment pheomelanins. Pigment Cell Res. 16(5):532-9, 2003.

- Ye T, Lamb LE, Wakamatsu K, Ito S, Simon JD.
  Ultrafast absorption and photothermal studies of decarboxytrichochrome C in solution. Photochem Photobiol Sci. 2(7):821-3, 2003.

- Ye T, Simon JD.
  The action spectrum for generation of the primary intermediate revealed by ultrafast absorption spectroscopy studies of pheomelanin. Photochem Photobiol. 77(1):41-5, 2003.

OTHER PIGMENTS

- Doss RP, Deisenhofer J, Krug von Nidda HA, Soeldner AH, McGuire RP.
  Melanin in the extracellular matrix of germlings of Botrytis cinerea. Phytochemistry.;63(6):687-91, 2003.

- Gomez BL, Nosanchuk JD.
  Melanin and fungi. Curr Opin Infect Dis. 16(2):91-6, 2003.

- Hung YC, Sava VM, Blagodarsky VA, Hong MY, Huang GS.
  Protection of tea melanin on hydrazine-induced liver injury. Life Sci. 72(9):1061-71, 2003.

- Ide F, Mishima K, Saito I.
  Melanin pigmentation in the juxtaoral organ of Chievitz. Pathol Int. 53(4):262-3, 2003.

- Ikeda R, Sugita T, Jacobson ES, Shinoda T.
  Effects of melanin upon susceptibility of Cryptococcus to antifungals. Microbiol Immunol. 47(4):271-7, 2003.

- Langfelder K, Streibel M, Jahn B, Haase G, Brakhage AA.
  Biosynthesis of fungal melanins and their importance for human pathogenic fungi. Fungal Genet Biol. 38(2):143-58, 2003.

- Lanisnik Rizner T, Wheeler MH.
  Melanin biosynthesis in the fungus Curvularia lunata (teleomorph: Cochliobolus lunatus). Can J Microbiol. 49(2):110-9, 2003.

- Morris-Jones R, Youngchim S, Gomez BL, Aisen P, Hay RJ, Nosanchuk JD, Casadevall, A, Hamilton AJ
  Synthesis of melanin-like pigments by Sporothrix schenckii in vitro and during mammalian infection. Infect Immun. 71(7):4026-33, 2003.

- Vogliardi S, Allegri G, Bertazzo A, Costa CV, Seraglia R, Traldi P.
  An investigation on the role of 5-hydroxytryptophan in the biosynthesis of melanins. J Mass Spectrom. 37(12):1292-6, 2002.

2. Biology of pigment cells and pigmentary disorders

(Dr. M. Picardo)

Kadekaro et al extensively reviewed the current state of knowledge about cutaneous photobiology. The role of melanogenesis as photoprotective response was analysed in detail considering the contributions of the absolute content of melanin, the distribution of melanosomes, the relative amounts of eumelanin and pheomelanin to the extent and efficacy of skin defence against UV. Furthermore the association between increased melanogenesis, amount of DNA damage and DNA repair was reported as possible explanation for the variation in the extent of mutagenesis and skin cancer risk among individuals with different pigmentary phenotype and tanning ability. The data concerning the investigations on the signalling pathways of UV in melanocytes are well organised and focused on the connections between the different parameters of cell cycle progression and survival/death signals: with accumulation of p53, p21, p16, hypo phosphorylation of RB, ratio between Bcl-2 and Bax expression, activation of the death receptors Fas (CD95) or TNF-α receptor 1, activation of the IP3kinase-Akt pathway which the subsequent activation of Mitf and expression of tyrosinase and TRP-1 genes. Moreover, strong evidences of a link between the p53 pathway and melanogenic response, which confers phototoprotection, have been reported. Also all the correlations between paracrine factors and genetic background in the induction of melanogenesis have been clearly exposed.

The role of melanin, or other factors selectively found in pigmented melanocytes in protection against UVA-induced lipid and membrane damage was investigated by Kvam and Dahle. Employing three mouse melanocyte cell lines, with different degree of pigmentation, the authors showed that pigmented melanocytes are more resistant to UVA-induced lipid peroxidation and membrane damage than non-pigmented melanocytes. The deficit of cellular antioxidants and the absence of melanin have been suggested to play a key role in determining the high susceptibility of unpigmented melanocytes to UVA irradiation harmful effects. Effectively, the selective depletion, by buthionine sulfoximine treatment, of glutathione was found to enhance the lipid peroxidation only in non-pigmented melanocytes, suggesting the presence inside pigmented melanocytes of other antioxidants able to protect the cells. These results are in agreement with data provide by other authors reporting an imbalance between antioxidants and peroxidative targets in melanocytes from subjects with low phototype. Moreover, the induction of melanin synthesis by tyrosine treatment was showed to determine only a weak increase of lipid peroxidation after UVA exposure in pigmented melanocytes, suggesting that the basal content of melanin could be sufficient to inhibit damage and that the eumelanin precursors have weak prooxidant properties.

Parsad and co-workers analysed the relative proportion of pheomelanin and eumelanin in both depigmented and repigmented skin of vitiligo patients following PUVA therapy. They found in depigmented lesions a higher content of pheomelanin, whereas in the repigmented lesions a predominant amount of eumelanin was detected. The authors suggested that the activation of a complex sequence of events leading to death or inactivation of melanocytes, deficit of tyrosinase activity and subsequent switch to pheomelanin synthesis could play a role in vitiligo pathogenesis. The high concentration of eumelanin in PUVA-treated repigmented is explained as a consequence of the activation of melanocyte in the outer root sheath of the hair follicle.

The in vitro models of melanocytes behaviour are usually utilized to understand the physiological and pathological activities in vivo. However, the artifious condition of the cell culture with respect to the in vivo life is hardly affected by medium composition and techincal procedures. Luckily, sometime the researchers present clear and intersting data on the influence of the in vitro condition on the cell activities and response. Obviously, these informations help the other lab investigators and allow a better understand of the cell physiopathology. At this regard, T Hirobe in two different papers published by Pigment Cell Res evaluated the role of the Steel Factor (SLF) and tyrosine on melanocyte proliferation and differentiation. He used different culture media and melanocytes and melanoblasts cultures. SLF induce the formation of dendritic processes in melanomlasts and the differentiation in melanocytes. It stimulates the progression from Go/G1 or G2 to M phase of the cel cycle and attends to the proliferation and differentiation in cooperation with cAMP, -MSH and bFGF. The soluble form of SLF is produced by keratinocytes and acts as regulator of proliferation and differentiation of the melanocytes. The involvement of cAMP and bFGF suggests a role for the protein kinase A and C in these processes. The other paper of Hirobe illustrates the role of L-Tyrosine in proliferation and differentiation of melanoblasts and melanocytes. In mouse pink eyed (p/p) melanocytes and melonoblasts an excess of L-Tyrosine affects the proliferation rate. Probably, in p/p melanocytes there is a defect of accumulation of eu- and pheomelanin and not a defect of the tyrosinase patway. Frequently, the colture of primary melanocytes exhibits the risk of a contamination by other cell types, such as fibroblasts and keratinocytes. The SA Leachman group elaborates a method to characterize melanocytic population in colture. They translate a hystopathology method (FCB method) to the cell colture sphere. The procedure requires a small amount of cells and is easy and fast, without doubt. However, the comparison proposed provides some doubts. The flow cytometric method needs of more cells with respect to the FCB but it allows also, in our opinion, a morphological evaluation and a discrimination between melanocyes, fibroblasts and keratinocytes based on physical parameters, permits the generation of an electronic archive, and the reutilization of the characterized cells by means of the sorting device.

The molecular mechanisms underlying the pigmentation are continuously studied. M Khaled illustrates the link between MITF activation and PI3K pathway: the increased expression of tyrosinase and TRP1 caused by PI3K inhibition depends on a transcriptional mechanism through distal regulatory elements. The PI3K inhibition enhances the activity of the glycogen synthase kinase 3 with a subsequent phosphorylation of -catenin. -catenin in turn regulates Mitf transcription. Gaggioli et al demonstrated that MITF is required but does not seem sufficient to induce the expression of tyrosinase and Tyrp1. These results seem to disagree with previous data reporting that MITF is a key component of the cAMP cascade leading to the stimulation of melanogenic enzymes. However the authors also clearly demonstrated that the

inhibition of endogenous MITF blocks the expression of tyrosinase and Tyrp1 in B16 melanoma cells and in human melanocytes. Thus, the existence of a still unknown regulatory mechanism, that is involved together with MITF in controlling melanogenic gene expression and melanin synthesis, has been hypothesized.

What appens to the melanocytes during vitiligo process and therapeutical procedures? These questions represent ground zero of the work for several groups. IC Le Poole, a historical researcher around vitiligo, discussed the problem by the immunological/genetic perspective and indicated a reduced expression of CDw60 associated with an increased amount of the HLA-DR in the perilesional areas. The relevance of the CDw60 cames from its identification as the ganglioside GD3, involved, as inhibitor of NF- B, in the apoptotic pathway. At this regards, we propose (see below) also a short bibliography on "GD3 & apoptosis", considering the possible crucial role of this mechanism in the melonocytes disappearance. H Kemp carries on the evaluation of the occurrence of autoantibodies against MCHR1. MCH has been reported to counteract the action of -MSH. In this paper he studies the B cell epitopes on MCHR1 recognized by autoantibodies in vitiligo patients. The epitope characterization could explain the heterogeneity of the immune response and help in the developing of a diagnostic assay. On the other hand, G Carlie suggests a possible mechanism of repigmentation KUVA-associated. In a in vitro model, he found that khellin improves both the proliferation and the melanin syntheis induced by UVA irradiation. The last effect appears to be due to an incread glicosylation of the enzymes (tyrosinase and DCT) involved in the melanogenesis. However, a larger amount of khellin was found toxic for the cells, suggesting that a fine management of the in vivo khellin application needs. The involvement of the T lymphocytes in the pathogenesis of the vitiligo renders interesting a review article published by the J Inv Dermatol and wroten by MP Schon. The author reflects on the lymphocytes recruitment at the inflammation sites and presents a punctual and precious description of the different phases of the process. Even if the paper appears to be of immunological pertinence, it can be helpful for a better knowledge of some immune-mediated skin diseases.

Kim et al showed that exposure to low temperature and the duration of this exposure are relevant regulators of melanogenesis. They demonstrated that a mouse melanocyte cell line or human primary melanocytes cultured at low temperature produced less melanin than cells at 37°C.

The different response induced in melanocyte following UVA or UVB has been investigated from Abdel-Naser and co-workers. Following exposure of normal human melanocytes to a single UVA dose only minimal effects, without changes in morphology, cell number, melanin synthesis and antigen expression, were observed. On the other hand, UVB radiation significantly induced dendrite formation and decreased the melanocyte number in a dose-dependent manner. Thus, the authors suggested that the effect of UVB radiation is due to direct activation of melanocytes, whereas skin tanning caused by UVA is mediated rather in an indirect way.

Tyrosine phosphorilation is reguleted by protein tyrosine kinases and phosphatases. The first ones mediated the addition and the second ones the removal of phosphate groups from protein substrates. Tyrosine kinases are considered as oncogenes, posphatases have been hypothesized to function as tumour uppressor genes. Mc Ardle et al. try to investigate possible changes in tyrosine phosphate and tyrosine phosphatases activities occurring in melanoma progression. Immunohistochemistry was used to study phosphotyrosine in melanocytic lesions. In addition, in normal melanocytes and melanoma cell lines phosphotase activity was measured by using an enzyme-linked immunosorbed assay-based system. Normal melanocytes, most benig naevi and melanoma to early phases, were negative after immunostaining. On the contrary, most advanced melanomas were immunoreactive. These results show that increased phosphotyrosine signalling occurs during melanoma progression at the stage when cells first become ompetent for metastasis.

Quast and co-workers showed the high expression of β-amyloid precursor protein (sAPP) in epidermal melanocytes and melanoma cells and its applicability as a marker protein for this cell type. However, APP is not a selective melanocyte marker as it is expressed at much higher levels in melanocytes and also found in keratinocytes. In this respect, APP shares features with other useful but non-specific melanocyte markers such as MC1-R. Moreover the authors demonstrated that, similar to α-MSH, sAPP operates as a potent regulator of melanocyte motility and melanin release at dendritic tips.

Julé et al. established and characterized a non-tumorigenic immortalized cell line of normal pigment melanocyte, derived from pig skin. This cell line, that was designated as PigMel, presents some characteristics such as differentiation functions in culture, dependence on TPA and cholera toxin and presents a diploid chromosome number. Moreover, PigMel cells shows morphological and molecular characteristics tipical of normal mammalian skin melanocytes. In monolayer culture, Pig Mel, are triangular or fusiform with moderate dendritic morphology and contain melanin in their cytoplasm. PigMel remained dependent on FCS for proliferation and developed no multilayer foci at confluence, and did not proliferate anchorage-independently in soft agar. Moreover PigMel cells did not prove to be tumorigenic after intradermal injection into athymic mice.

Morphologic features and growth properties of PigMel cells are suggestive of an immortalized but not tumorigenic cell line.

The expression of inducible nitric oxide synthase in melanoma tumor cells, was recently shown to correlate strongly with poor patients survival after combination biochemoterapy. Furthermore, evidence suggest that nitric oxide, a reaction product of nitric oxyde synthase, exhibits antiapoptotic activity in melanoma cells. Tang and Grimm hypothesized that nitric oxide antagonizes the chemotherapy-induced apoptosis. For this reason they analysed whether nitric oxide is capable of regulating cell growth and apoptotic response to cisplatin treatment in melanoma cell lines and demonstrated that the depletion of endogenously produced nitric oxide is required for cisplatin-induced p53 activation and p21 expression, wich can regulate melanoma sensitivity to cisplatin.

By proteomic analysis, Bernard et al. identified eight candidate markers of melanoma progression as differentially regulated in transformed cells. In particular, Hepatoma-derived growth factor (EDGF) and nucleophosmin B23 resulted strongly correlated with melanoma. Western blot analysis in two dimensional gel showed that the form of nucleophosmin B23 that is up-regulated in melanoma, represent postraslationally modified form, most likely reflecting enhanced phosphorylation in the tumor-derived cells. In contrast, Western analysis of HDGF demonstrated increased expression of all forms in melanoma cells compared with melanocytes. Immunohistochemical analysis of human tissue biopsies showed a strong expression of HDGF in early and late stage melanomas and low expression in melanocytes and nontumorigenic nevi. Interestingly, biopsies of nevi showed a graded effect in which HDGF immunoreactivity resulted lower in nevoid nests penetrating deep into the dermis in comparison with nests at the epidermal-dermal junction, suggesting that HDGF expression in nevi is dependent on the epidermal cell interactions. In contrast, biopsies of melanoma showed strong expression of HDGF throughout the tumor, including cells located deeply within dermis.

- Abdel-Naser MB, Krasagakis K, Garbe C, Eberle J.
  Direct effects on proliferation, antigen expression and melanin synthesis of cultured normal human melanocytes in response to UVB and UVA light. Photodermatol Photoimmunol Photomed 19: 122-127, 2003.

- Bernard K, Litman E, Fitzpatrick JL, Shellman YG, Arganst G, Polvi K, Everett AD, Fukasawa K Norris DA, Ahn NG, Resing KA.
  Functional proteomic analysis of melanoma progression. Cancer Res 63: 6716-6725, 2003.

- Carlie G, Antusi NB, Hulley PA, Kidson SH.
  KUVA (khellin plus ultraviolet A) stimulates proliferation and melanogenesis in normal human melanocytes and melanoma cells in vitro. Br J Dermatol 149: 707-17, 2003.

- Florell SR, SJ Schmidt, P Porter-Gill, KH Albertine, KJ Murphy, CB Mckinney, KM Boucher, D Grossman, DL Biddle, F Clayton, LJ Layfield, SA Leachman.
  Novel application of a fibrin cell block method to evaluate melanocytic cell populations. Pigment Cell Res 16: 662-9, 2003.

- Gaggioli C, Busca R, Abbe P, Ortonne JP, and Ballotti R.
  Microphtalmia-associated transcription factor (MITF) is required but is not sufficient to induce the expression of melanogenic genes. Pigment Cell Res 16:374-82, 2003.

- Gottumukkala RVSRK, Waterman EA, Herd LM, Gawkrodger DJ, Watson PF, Weetman AP, Kemp EH.
  Autoantibodies in vitiligo patients recognize multiple domains of the melanin-concentrating hormone receptor. J Invest Dermatol 121: 765-70, 2003.

- Hirobe T, Wazumasa K, Ito S.
  Changes in the proliferation and differentiation of neonatal mouse pink-eyed dilution melanocytes in the presence of excess tyrosine. Pigment Cell Res 16: 619-28, 2003.

- Hirobe T, Osawa M, Nishikawa SI.
  Steel factor controls the proliferation and differentiation of neonatal mouse epidermal melanocytes in culture. Pigment Cell Res 16: 644-55, 2003.

- Kadekaro AL, Kavanagh RJ, Wamatsu K, Ito S, Pipitone MA and Abdel-Malek ZA**.**
  Cutaneous photobiology. The melanocyte vs. sun: who will win the final round? Pigment Cell Res 16: 434-447, 2003.

- Khaled M, Larribere L, Bille K, Ortonne JP, Ballotti R, Bertolotto C.
  Microphtalmia associated transcription factor is a target of the phosphatidylinositol-3-kinase pathway. J Invest Dermatol 121: 831-6, 2003.

- Kim DS, Park SH, Kwon SB, Joo YH, Youn SW, Sohn UD and Park KC.
  Temperature regulates melanin synthesis in melanocytes. Arch Pharm Res 26: 840-845, 2003.

- Kvam E and Dahle J.
  Pigmented melanocytes are protected against ultraviolet-A-induced membrane damage. J Invest Dermatol 121: 564-569, 2003.

- Julé S, Bossé P, Egidy G, Panthier JJ.
  Establishment and characterization of a normal melanocyte cell line derived from pig skin. Pigment Cell Res 16: 407-410, 2003.

- Le Poole IC Stennett , LS, BK Bonish, L Dee, JK Robinson, C Hernandez, SK Hann, BJ Nickoloff.
Expansion of vitiligo lesions is associated with reduced epidermal CDw60 expression and increased expression of HLA-DR in perilesional skin. Br J Dermatol 149: 739-48, 2003.

- McArdle L, Bergin O, Fallowfield ME, Dervan PA, Easty DJ.
Tyrosine phosphate in melanoma progression. Br J Dermatol 149: 289-295, 2003.

- Parsad D, Wakamatsu K, Kanwar AJ, Kumar B and Ito S.
Eumelanin and pheomelanin contents of depigmented and repigmented skin in vitiligo patients. Br J Dermatol 149: 624-626, 2003.

- Quast T, Wehner Sven, Kirfel G, Jaeger K, De Luca M and Herzog V.
sAPP as a regulator of dendrite motility and melanin release in epidermal melanocytes and melanoma cells. FASEB J 17: 1739-1741, 2003.

- Schon MP, Zollner TM, Henning Boehncke W.
The molecular basis of lymphocyte recruitment to the skin: clues for pathogenesis and selective therapies of inflammatory disorders. J Invest Dermatol 121: 951-62, 2003.

- Tang CH and Grimm EA.
Depletion of endogenous nitric oxide enhances cisplatin-induced apoptosis in a p53-dependent manner in a melanoma cell line. J Biol Chem. Oct 23 [Epub ahead print]2003.

- Wakamatsu K, Yokochi M, Naito A, Kageshita T and Ito S.
Comparison of pheomelanin and its precursor 5-S-cysteinyldopa in the serum of melanoma patients. Melanoma Res 13:357-363, 2003.

GD3 & apoptosis:

- Colell A, Garcia-Ruiz C, Roman J, Balestra A, Fernandez-Checa JC.
Ganglioside GD3 enhances apoptosis by suppressing the nuclear factor-kB-dependent survival pathway. FASEB J 15: 1068-70, 2001.

- Colell A, Morales A, Fernandez-Checa JC, Garcia-Ruiz C.
Ceramide generated by acidic sphingomyelinase contributes to tumor necrosis factor- -mediated apoptosis in human colon HT-29 cells through glycosphingolipids formation. FEBS Lett 526:135-41, 2002.

- Garcia-Ruiz C, Colell A, Morales A, Calvo M, Enrich C, Fernandez-Checa JC.
Trafficking of ganglioside GD3 to mitochondria by tumor necrosis factor- . J Biol Chem 277(39): 36443-8, 2002.

- Malisan F, Franchi L, Tomassini B, Ventura N, Condò I, Rippo MR, Rufini A, Liberati L, Nachtigall C, Kniep B, Testi R.
Acetylation suppresses the proapoptotic activity of GD3 ganglioside. J Exp Med 196(12): 1535-41, 2002.

- Rippo MR, Malisan F, Ravagnan L, Tomassini B, Condo I, Costantini P, Susin SA, Rufini A, Todaro M, Kroemer G, Testi R.
GD3 ganglioside directly targets mitochondria in a bcl-2 controlled fashion. FASEB J 14: 2047-54, 2000.

Bibliography not discussed:

- Arroyo MP, Tift L.
Vitiligo therapy: where are we now? J Drug Dermatol 2(4): 404-8, 2003.

- Casp CB, She JX, McCormack WT.
Genes of the LMP/TAP cluster are associated with the human autoimmune disease vitiligo. Genes Immun 4(7): 492-9, 2003.

- Cheung M, Briscoe J.
Neural crest development is regulated by the transcription factor Sox9. Development 130(23): 5681-93, 2003.

- Conner SR, Scott G, Aplin AE.

Adhesion-dependent activation of the ERK1/2 cascade is by-passed in melanoma cells. J Biol Chem 278 (36): 34548-54, 2003.

- Eves P, Haycock J, Layton C, Wagner M, Kemp H, Szabo M, Morandini R, Ghanem G, Garcia-Borron JC, Jimenez-Cervantes C, Mac Neil S.
  Anti-inflammatory and anti-invasive effects of alpha-melanocyte-stimulating hormone in human melanoma cells. Br J Cancer 89(10): 2004-15, 2003.

- Guerra L, Primavera G, Raskovic D, Pellegrini G, Molisano O, Bondanza S, Paterna P, Sonego G, Gabello T, Attori F, Piazza P, Luci A, De Luca M.
  Erbium: YAG laser and cultured epidermis in the surgical therapy of stable vitiligo. Arch Dermatol 139(10): 1303-10, 2003.

- Huber WE, Price ER, Widlund HR, Du J, Davis IJ, Wegner M, Fisher DE.
  A tissue restricted cAMP transcriptional response: SOX10 modulates alpha-melanocyte-stimulating hormone triggered expression of microphtalmia-associated transcription factor in melanocytes. J Biol Chem 278(46): 45224-30, 2003.

- Kim DS, ES Hwang, JE Lee, SY Kim, KC Park.
  Sphingosine-1-phosphate promotes mouse melanocyte survival via ERK and Akt activation. Cell Signal 15(10): 919-26, 2003.

- Mandelcorn-Monson RL, Shear NH, Yau E, Sambhara S, Barber BH, Spanner D, DeBenedette MA.
  Cytotoxic T lymphocyte reactivity to gp100, MelanA/MART1, and tyrosinase in HLA-A2-positive vitiligo patients. J Invest Dermatol 121(3): 550-6, 2003.

- Mantovani S, Garbelli S, Palermo B, Campanelli R, Barzelli V, Borroni G, Martinetti M, Benvenuto F, Merlini G, Della Cuna GR, Rivoltini L, Giachino C.
  Molecular and functional bases of self-antigen recognition in long-term persistent melanocyte-specific-CD8+ T cells in one vitiligo patient. J Invest Dermatol 121(2): 308-14, 2003.

- Wang X, Erf GF.
  Melanocyte-specific cell mediated immune response in vitiliginous smyth line chickens. J Autoimmun 21(2): 149-60, 2003.

2. MSH, MCH, other hormones, differentiation

(Dr. R. Morandini)

Regulation and signal transduction

- Galibert M, Corre S, Primot A.
  SP-19 Molecular mechanism upon UV-irradiation of the alphaMSH and MC1R genes. Pigment Cell Res. 16(5):587, 2003.

- Garcia-Borron JC.
  IL-03 Biochemical aspects of MC1R signalling through the cAMP pathway. Pigment Cell Res. 16(5):570-1, 2003.

- Getting SJ, Schioth HB, Perretti M.
  Dissection of the anti-inflammatory effect of the core and C-terminal (KPV) alpha-melanocyte-stimulating hormone peptides. J Pharmacol Exp Ther. 306(2):631-7, 2003.

- Hata K, Hori K, Takahashi S.
  Role of p38 MAPK in Lupeol-Induced B16 2F2 Mouse Melanoma Cell Differentiation. J Biochem (Tokyo). 134(3):441-5, 2003.

- Laurent V, Jaubert-Miazza L, Desjardins R, Day R, Lindberg I.
  Biosynthesis of POMC-derived peptides in prohormone convertase 2 and 7B2 null mice. Endocrinology, 2003.

- Mas JS, Gerritsen I, Hahmann C, Jimenez-Cervantes C, Garcia-Borron JC.
  Rate limiting factors in melanocortin 1 receptor signalling through the cAMP pathway. Pigment Cell Res. 16(5):540-7, 2003.

- Ohguchi K, Banno Y, Akao Y, Nozawa Y.
Involvement of phospholipase D1 in melanogenesis of mouse B16 melanoma cells. J Biol Chem. 2003.

- Rouzaud F, Annereau JP, Valencia JC, Costin GE, Hearing VJ.
Regulation of melanocortin 1 receptor expression at the mRNA and protein levels by its natural agonist and antagonist. FASEB J. 17(14):2154-6, 2003.

- Sarkar A, Sreenivasan Y, Manna SK.
alpha-Melanocyte-stimulating hormone inhibits lipopolysaccharide-induced biological responses by downregulating CD14 from macrophages. FEBS Lett. 553(3):286-94, 2003.

- Voisey J, Carroll L, van Daal A.
Melanocortins and their receptors and antagonists. Curr Drug Targets. 4(7):586-97. Review, 2003.

- Wachira SJ, Hughes-Darden CA, Taylor CV, Ochillo R, Robinson TJ.
Evidence for the interaction of protein kinase C and melanocortin 3-receptor signaling pathways. Neuropeptides. 37(4):201-10, 2003.

- Yoon SW, Goh SH, Chun JS, Cho EW, Lee MK, Kim KL, Kim JJ, Kim CJ, Poo H.
alpha-Melanocyte-stimulating hormone inhibits lipopolysaccharide-induced tumor necrosis factor-alpha production in leukocytes by modulating protein kinase A, p38 kinase, and nuclear factor kappa B signaling pathways. J Biol Chem. 278(35):32914-20, 2003.

Effect on cell *in vitro*

- Bohm M, Luger TA.
IL-30 UV-mediated induction of melanocortins in the skin. Pigment Cell Res. 16(5):586-7, 2003.

- Canton I, Eves PC, Szabo M, Vidal-Vanaclocha F, Sisley K, Rennie IG, Haycock JW, MacNeil S.
Tumor necrosis factor alpha increases and alpha-melanocyte-stimulating hormone reduces uveal melanoma invasion through fibronectin. J Invest Dermatol. 121(3):557-63, 2003.

- Catania A, Colombo G, Carlin A, Garofalo L, Gatti S, Buffa R, Carboni N, Rosso L, Santambrogio L, Cantalamessa L, Lipton JM.
Autocrine inhibitory influences of {alpha}-melanocyte-stimulating hormone in malignant pleural mesothelioma. J Leukoc Biol. 2003.

- Eves P, Haycock J, Layton C, Wagner M, Kemp H, Szabo M, Morandini R, Ghanem G, Garcia-Borron JC, Jimenez-Cervantes C, Mac Neil S.
Anti-inflammatory and anti-invasive effects of alpha-melanocyte-stimulating hormone in human melanoma cells. Br J Cancer. 89(10):2004-15, 2003.

- Eves P, Kemp EH, Morandini R, Ghanem G, Garcia-Barron JC, Jimenez-Cervantes C, Haycock JW, Mac Neil S.
PP-15 Alpha-melanocyte stimulating hormone (alpha-MSH) and melanoma invasion: introduction of melanocortin wild-type receptor (MC-1R) into previously alpha-MSH unresponsive cells leads to alpha-MSH inhibition of invasion in transfected cells. Pigment Cell Res. 16(5):597-8, 2003.

- Fernandez RM, Ito AS, Schioth HB, Lamy MT.
Structural study of melanocortin peptides by fluorescence spectroscopy: identification of beta-(2-naphthyl)-D-alanine as a fluorescent probe. Biochim Biophys Acta. 1623(1):13-20, 2003.

- Jimenez-Cervantes C, Sanchez J, Gerritsen I, Hahmann C, Garcia-Borron JC.
SP-02 Agonist-independent, high constitutive activity of the human melanocortin 1 receptor. Pigment Cell Res. 16(5):571-2, 2003.

- Huber WE, Price ER, Widlund HR, Du J, Davis IJ, Wegner M, Fisher DE.
A tissue-restricted cAMP transcriptional response: SOX10 modulates alpha-melanocyte-stimulating hormone-triggered expression of microphthalmia-associated transcription factor in melanocytes. J Biol Chem 278(46):45224-30, 2003.

- Matsumura R, Takagi C, Kakeya T, Okuda K, Takeuchi S, Takahashi S.
Alpha-melanocyte-stimulating hormone stimulates prolactin secretion through melanocortin-3 receptors expressed in mammotropes in the mouse pituitary. Neuroendocrinology. 78(2):96-104, 2003.

- Miao Y, Whitener D, Feng W, Owen NK, Chen J, Quinn TP.
Evaluation of the Human Melanoma Targeting Properties of Radiolabeled alpha-Melanocyte Stimulating Hormone Peptide Analogues. Bioconjug Chem. 14(6):1177-84, 2003.

- Mockenhaupt M, Peters F, Schwenk-Davoine I, Herouy Y, Schraufstatter I, Elsner P, Norgauer J.
Evidence of involvement of CXC-chemokines in proliferation of cultivated human melanocytes. Int J Mol Med. 12(4):597-601, 2003.

- Sabatier N, Caquineau C, Dayanithi G, Bull P, Douglas AJ, Guan XM, Jiang M, Van der Ploeg L, Leng G.
Alpha-melanocyte-stimulating hormone stimulates oxytocin release from the dendrites of hypothalamic neurons while inhibiting oxytocin release from their terminals in the neurohypophysis. J Neurosci 23(32):10351-8, 2003.

- Sarkar A, Sreenivasan Y, Manna SK.
alpha-Melanocyte-stimulating hormone induces cell death in mast cells: involvement of NF-kappaB. FEBS Lett. 14;549(1-3):87-93, 2003.

- Zanna P, Guida G, Gallone A, Argenzio E, Boffoli D, Cicero R.
PP-12 Kupffer Cell response to MSH in Rana esculenta L. Pigment Cell Res. 16(5):596-597, 2003.

Clinical investigations

- Eberle AN, Froidevaux S.
Radiolabeled alpha-melanocyte-stimulating hormone analogs for receptor-mediated targeting of melanoma: from tritium to indium. J Mol Recognit. 16(5):248-54, 2003.

- Gottumukkala RV, Waterman EA, Herd LM, Gawkrodger DJ, Watson PF, Weetman AP, Kemp EH.
Autoantibodies in vitiligo patients recognize multiple domains of the melanin-concentrating hormone receptor. J Invest Dermatol. 121(4):765-70, 2003.

- Gottumukkala R, Gawkrodger DJ, Watson PF, Weetman AP, Kemp EH.
PP-27 B cell epitope mapping of the vitiligo autoantigen melanin-concentrating hormone receptor. Pigment Cell Res. 16(5):602, 2003.

- Mioni C, Giuliani D, Cainazzo MM, Leone S, Iannone C, Bazzani C, Grieco P, Novellino E, Tomasi A, Bertolini A, Guarini S.
Further evidence that melanocortins prevent myocardial reperfusion injury by activating melanocortin MC3 receptors. Eur J Pharmacol. 477(3):227-34, 2003.

- Tao YX, Segaloff DL.
Functional characterization of melanocortin-4 receptor mutations associated with childhood obesity. Endocrinology. 144(10):4544-51, 2003.

- Verret L, Goutagny R, Fort P, Cagnon L, Salvert D, Leger L, Boissard R, Salin P, Peyron C, Luppi PH.
A role of melanin-concentrating hormone producing neurons in the central regulation of paradoxical sleep. BMC Neurosci. 4(1):19, 2003.

- Willems M, Munte K, Vrolijk JM, Den Hollander JC, Bohm M, Kemmeren MH, De Man RA, Brouwer JT.
Hyperpigmentation during interferon-alpha therapy for chronic hepatitis C virus infection. Br J Dermatol. 149(2):390-4, 2003.

Others - Not classified

- Abbott CR, Kennedy AR, Wren AM, Rossi M, Murphy KG, Seal LJ, Todd JF, Ghatei MA, Small CJ, Bloom SR.
Identification of hypothalamic nuclei involved in the orexigenic effect of melanin-concentrating hormone. Endocrinology. 144(9):3943-9, 2003.

- Cerda-Reverter JM, Schioth HB, Peter RE.
The central melanocortin system regulates food intake in goldfish. Regul Pept. 115(2):101-13, 2003.

- Chaki S, Ogawa S, Toda Y, Funakoshi T, Okuyama S.
Involvement of the melanocortin MC4 receptor in stress-related behavior in rodents. Eur J Pharmacol. 474(1):95-101, 2003.

- Davidowa H, Li Y, Plagemann A.
Altered responses to orexigenic (AGRP, MCH) and anorexigenic (alpha-MSH, CART) neuropeptides of paraventricular hypothalamic neurons in early postnatally overfed rats. Eur J Neurosci. 18(3):613-21,.2003.

- Gao XB, Prabhat GK, Van Den Pol AN.
Neurons synthesizing melanin concentrating hormone identified by selective reporter gene expression after transfection in vitro: transmitter responses. J Neurophysiol, 2003.

- Hansen SL, Fjalland B, Jackson MB.
Modulation of GABAA receptors and neuropeptide secretion by the neurosteroid allopregnanolone in posterior and intermediate pituitary. Pharmacol Toxicol. 93(2):91-7, 2003.

- Healy E, Robinson SJ, Dixon SV.
IL-13 Alpha-melanocyte stimulating hormone; a matter of life and death. Pigment Cell Res16(5):577, 2003.

- Hervieu G.
Melanin-concentrating hormone functions in the nervous system: food intake and stress. Expert Opin Ther Targets. 7(4):495-511, 2003.

- Itateyama E, Chiba S, Sakata T, Yoshimatsu H.
Hypothalamic neuronal histamine in genetically obese animals: its implication of leptin action in the brain. Exp Biol Med (Maywood). 228(10):1132-7, 2003.

- Lavallee CR, Chalifoux JR, Moosally AJ, Balkema GW.
Elevated free calcium levels in the sub-retinal space elevate the absolute dark-adapted threshold in hypopigmented mice. J Neurophysiol. 2003.

- Lio P, Vannucci M.
Investigating the evolution and structure of chemokine receptors. Gene. 317:29-37, 2003.

- Lu XY, Barsh GS, Akil H, Watson SJ.
Interaction between alpha-melanocyte-stimulating hormone and corticotropin-releasing hormone in the regulation of feeding and hypothalamo-pituitary-adrenal responses. J Neurosci. 23(21):7863-72, 2003.

- Miller R, Aaron W, Toneff T, Vishnuvardhan D, Beinfeld MC, Hook VY.
Obliteration of alpha-melanocyte-stimulating hormone derived from POMC in pituitary and brains of PC2-deficient mice. J Neurochem. 86(3):556-63, 2003.

- Naveh N.
Melanocortins applied intravitreally delay retinal dystrophy in Royal College of Surgeons rats. Graefes Arch Clin Exp Ophthalmol. 2003.

- Niederkorn JY.
Mechanisms of immune privilege in the eye and hair follicle. J Investig Dermatol Symp Proc8(2):168-72, 2003.

- O'Shaughnessy PJ, Fleming LM, Jackson G, Hochgeschwender U, Reed P, Baker PJ.
Adrenocorticotropic hormone directly stimulates testosterone production by the fetal and neonatal mouse testis. Endocrinology. 144(8):3279-84, 2003.

- Pereira-da-Silva M, Torsoni MA, Nourani HV, Augusto VD, Souza CT, Gasparetti AL, Carvalheira JB, Ventrucci G, Marcondes MC, Cruz-Neto AP, Saad MJ, Boschero AC, Carneiro EM, Velloso LA.
Hypothalamic melanin-concentrating hormone is induced by cold exposure and participates in the control of energy expenditure in rats. Endocrinology. 144(11):4831-40, 2003.

- Phillips-Singh D, Li Q, Takeuchi S, Ohkubo T, Sharp PJ, Boswell T.
Fasting differentially regulates expression of agouti-related peptide, pro-opiomelanocortin, prepro-orexin, and vasoactive intestinal polypeptide mRNAs in the hypothalamus of Japanese quail. Cell Tissue Res313(2):217-25, 2003.

- Segal-Lieberman G, Bradley RL, Kokkotou E, Carlson M, Trombly DJ, Wang X, Bates S, Myers MG Jr, Flier JS, Maratos-Flier E.
Melanin-concentrating hormone is a critical mediator of the leptin-deficient phenotype. Proc Natl Acad Sci U S A. 100(17):10085-90, 2003.

- Shearman LP, Camacho RE, Sloan Stribling D, Zhou D, Bednarek MA, Hreniuk DL, Feighner SD, Tan CP, Howard AD, Van der Ploeg LH, MacIntyre DE, Hickey GJ, Strack AM.
Chronic MCH-1 receptor modulation alters appetite, body weight and adiposity in rats. Eur J Pharmacol. 475(1-3):37-47, 2003.

- Tobin DJ.
IL-09 Is beta-endorphin a melanotropin in the hair follicle pigmentary unit? Pigment Cell Res. 16(5):575-576, 2003.

- Vazquez-Martinez R, Castano JP, Tonon MC, Vaudry H, Gracia-Navarro F, Malagon MM.
Melanotrope secretory cycle is regulated by physiological inputs via the hypothalamus. Am J Physiol Endocrinol Metab. 285(5):E1039-46, 2003.

- Zou L, Sato N, Attuwaybi BO, Kone BC.
Delayed administration of alpha-melanocyte-stimulating hormone or combined therapy with BAY 11-7085 protects against gut ischemia-reperfusion injury. Shock. 20(5):469-75, 2003.

4. Photobiology

(Dr. N. Smit)

- Abdel-Naser MB, Krasagakis K, Garbe C, Eberle J.
Direct effects on proliferation, antigen expression and melanin synthesis of cultured normal human melanocytes in response to UVB and UVA light. Photodermatol.Photoimmunol.Photomed. 19:122-127, 2003.

- Bowen AR, Hanks AN, Allen SM, Alexander A, Diedrich MJ, Grossman D.
Apoptosis regulators and responses in human melanocytic and keratinocytic cells. J.Invest Dermatol. 120:48-55, 2003.
Melanocytes were relatively resistant to ultraviolet B, whereas keratinocytes were unresponsive to 4-tert-butylphenol. Melanocytes and keratinocytes were generally less susceptible than melanoma lines and HaCat cells to etoposide, cisplatin, and staurosporine. Induction of apoptosis in these cell types was generally associated with decreased levels of Mcl-1, XIAP, and Livin, and increased levels of p53, whereas levels of other apoptotic regulators were unaltered. These results provide insights into the potential roles of apoptosis in the function and transformation of epidermal melanocytes and keratinocytes.

- Byers HR, Maheshwary S, Amodeo DM, Dykstra SG.
Role of cytoplasmic dynein in perinuclear aggregation of phagocytosed melanosomes and supranuclear melanin cap formation in human keratinocytes. J.Invest Dermatol. 121:813-820, 2003.
Taken together, these findings indicate that in human keratinocytes, the retrograde microtubule motor cytoplasmic dynein mediates the perinuclear aggregation of phagocytosed melanosomes, participates in the formation of the supranuclear melanin cap or "microparasol" and serves as a mechanism to help protect the nucleus from ultraviolet-induced DNA damage.

- Cario-Andre M, Briganti S, Picardo M, Nikaido O, Gall Y, Ginestar J, Taieb A.
Epidermal reconstructs: a new tool to study topical and systemic photoprotective molecules. J.Photochem.Photobiol.B 68:79-87, 2002.

- Carlie G, Ntusi NB, Hulley PA, Kidson SH.
KUVA (khellin plus ultraviolet A) stimulates proliferation and melanogenesis in normal human melanocytes and melanoma cells in vitro. Br.J.Dermatol. 149:707-717, 2003.

- Girnita L, Girnita A, Larsson O.
Mdm2-dependent ubiquitination and degradation of the insulin-like growth factor 1 receptor. Proc.Natl.Acad.Sci.U.S.A 100:8247-8252, 2003.
In this study, we show that inhibition of p53 causes ubiquitination and down-regulation, through increased degradation, of the IGF-1R in human malignant melanoma cells. This effect, which was independent of the p53 status (i.e., wild type or mutated), was prevented if Mdm2 was coinhibited. Similar results were obtained in UV-irradiated human melanocytes (harboring wild-type p53), in which level of the IGF-1R increased after up-regulation of p53.
- Govindarajan B, Bai X, Cohen C, Zhong H, Kilroy S, Louis G, Moses M, Arbiser JL
Malignant transformation of melanocytes to melanoma by constitutive activation of mitogen-activated protein kinase kinase (MAPKK) signaling. J.Biol.Chem. 278:9790-9795, 2003.

- Ha T, Javedan H, Waterston K, Naysmith L, Rees JL.

The relationship between constitutive pigmentation and sensitivity to ultraviolet radiation induced erythema is dose-dependent. Pigment Cell Res. 16:477-479, 2003.

- Hiramoto K, Yanagihara N, Sato EF, Inoue M.
Ultraviolet B irradiation of the eye activates a nitric oxide-dependent hypothalamopituitary proopiomelanocortin pathway and modulates functions of alpha-melanocyte-stimulating hormone-responsive cells. J.Invest Dermatol. 120:123-127, 2003.

- Inoue K, Hosoi J, Ideta R, Ohta N, Ifuku O, Tsuchiya T.
Stress augmented ultraviolet-irradiation-induced pigmentation. J.Invest Dermatol. 121:165-171, 2003.

- Jhappan C, Noonan FP, Merlino G.
Ultraviolet radiation and cutaneous malignant melanoma. Oncogene 22:3099-3112, 2003.

- Kadekaro AL, Kavanagh RJ, Wakamatsu K, Ito S, Pipitone MA, Abdel-Malek ZA.
Cutaneous photobiology. The melanocyte vs. the sun: who will win the final round? Pigment Cell Res. 16:434-447, 2003.

- Kim DS, Kim SY, Lee JE, Kwon SB, Joo YH, Youn SW, Park KC.
Sphingosine-1-phosphate-induced ERK activation protects human melanocytes from UVB-induced apoptosis. Arch.Pharm.Res. 26:739-746, 2003.

- Kim TJ, Cho MK, Lee JS, Whang KU, Jin SY, Hoshino T.
The expression of melanogenic proteins in Korean skin after ultraviolet irradiation. J.Dermatol. 30:665-672, 2003.

- Kraus E, Galvin JW, Boumakis S, Boamah EK, Canning MT, Yarosh DB, Brown DA.
Effects of a melanogenic bicyclic monoterpene diol on cell cycle, p53, TNF-alpha, and PGE2 are distinct from those of UVB. Photodermatol.Photoimmunol.Photomed. 19:295-302, 2003.

- Kvam E, Dahle J.
Pigmented melanocytes are protected against ultraviolet-A-induced membrane damage. J.Invest Dermatol. 121:564-569, 2003.
Unpigmented cells were much more susceptible to ultraviolet-A-induced membrane permeability than pigmented cells. Unpigmented cells were also more susceptible to ultraviolet-A-induced lipid peroxidation than strongly pigmented cells. Furthermore, unpigmented cells were much more susceptible to ultraviolet-A-induced depletion of glutathione than pigmented cells.

- Lei TC, Vieira WD, Hearing VJ.
In vitro migration of melanoblasts requires matrix metalloproteinase-2: implications to vitiligo therapy by photochemotherapy. Pigment Cell Res. 15:426-432, 2002.

- Little EE, Calfee RD, Fabacher DL, Carey C, Blazer VS, Middleton EM.
Effects of ultraviolet radiation on toad early life stages. Environ.Sci.Pollut.Res.Int. 10:167-172, 2003.
B, breas tadpoles were more tolerant of simulated solar UVB exposure than B. woodhousii tadpoles, possibly because of greater amounts of photoprotective melanin in B. boreas skin.

- Marrot L, Belaidi JP, Jones C, Perez P, Riou L, Sarasin A, Meunier JR.
Molecular responses to photogenotoxic stress induced by the antibiotic lomefloxacin in human skin cells: from DNA damage to apoptosis. J.Invest Dermatol. 121:596-606, 2003.

- Schempp CM, Winghofer B, Muller K, Schulte-Monting J, Mannel M, Schopf E, Simon JC
Effect of oral administration ofHypericum perforatum extract (St. John's Wort) on skin erythema and pigmentation induced by UVB, UVA, visible light and solar simulated radiation. Phytother.Res. 17:141-146, 2003.

- Scott MC, Suzuki I, Abdel-Malek ZA.
Regulation of the human melanocortin 1 receptor expression in epidermal melanocytes by paracrine and endocrine factors and by ultraviolet radiation. Pigment Cell Res. 15:433-439, 2002.

- Stefanato CM, Yaar M, Bhawan J, Phillips TJ, Kosmadaki MG, Botchkarev V, Gilchrest BA.
Modulations of nerve growth factor and Bcl-2 in ultraviolet-irradiated human epidermis. J.Cutan.Pathol. 30:351-357, 2003.

CONCLUSIONS: This is the first in vivo study showing NGF to be present in melanocytes, as well as showing modulations of NGF and Bcl-2 in melanocytes, following solar-simulated UV irradiation.

- Tadokoro T, Kobayashi N, Zmudzka BZ, Ito S, Wakamatsu K, Yamaguchi Y, Korossy KS, Miller SA, Beer JZ, Hearing VJ.
  UV-induced DNA damage and melanin content in human skin differing in racial/ethnic origin. FASEB J. 17:1177-1179, 2003.
  The results show that after exposure to 1 MED of UV, the skin of subjects from all groups suffered significant DNA damage, and that increasing content of constitutive melanin inversely correlated with the amount of DNA damage. It is clear from these results that measured erythemal UV sensitivity of the skin (MED) is a more useful predictor of DNA photodamage than is racial/ethnic origin or skin phototype and that rates of DNA damage removal following UV radiation may be the critical determinant of the UV sensitivity (including predisposition to cancer) of the skin.

- Yamakoshi J, Otsuka F, Sano A, Tokutake S, Saito M, Kikuchi M, Kubota Y.
  Lightening effect on ultraviolet-induced pigmentation of Guinea pig skin by oral administration of a proanthocyanidin-rich extract from grape seeds. Pigment Cell Res. 16:629-638, 2003.

- Zhang H, Rosdahl I.
  Ultraviolet A and B differently induce intracellular protein expression in human skin melanocytes - a speculation of separate pathways in initiation of melanoma. Carcinogenesis 2003.
  The data suggested that UVA and UVB irradiation might lead to alterations of the different intracellular proteins. UVA enhanced protein expression concerning cell growth (p73 and Nup88) and UVB might overexpress proteins concerning cellular proliferation (Id1 and p27). UVA and UVB may induce initiation of melanoma via separate intracellular pathways.

5. Neuromelanins

(Prof. M. d'Ischia)

Research on the structure and pathophysiological roles of neuromelanin (NM) has been very active during 2003. Several reviews have become available (Ito et al., 2002; Matsunaga et al., 2002; Zecca et al., 2003a,b) that provide valuable up-to-date views of the state of the art in the field. Highlighted concepts include the following: a) the melanin moiety of neuromelanin consists mostly of dopamine-derived units with 10-20% incorporation of cysteinyldopamine-derived units; b) neuromelanin, rather than dopamine, plays a major role in the degeneration of nigral cells; c) macrophage inhibitory factor and DOPAchrome tautomerase may be involved as detoxifying enzymes in the process of neuromelanogenesis; d) intraneuronal neuromelanin could play a protective role during its synthesis but may cause toxicity under conditions of iron and toxin overload, inducing a vicious cycle of chronic neuroinflammation in Parkinson's disease.
These concepts are repeatedly emphasized in several research articles, a selected list of which is reported below. Wakamatsu et al. (2003) carried out alkaline hydrogen peroxide (H_2O_2) degaradation of neuromelanin and identified four degradation products, pyrrole-2,3-dicarboxylic acid (PDCA), pyrrole-2,3,5-tricarboxylic acid (PTCA), thiazole-4,5-dicarboxylic acid (TDCA) and thiazole-2,3,5-tricarboxylic acid (TTCA), whose ratios, especially the TTCA to PDCA ratio, indicates that NM is derived mostly from dopamine (DA) with 25% incorporation of cysteine (Cys) in the form of a benzothiazine structure. DOPA is not incorporated into NM to a significant extent (approximately 6% the level of DA). They propose that the TTCA to PDCA ratio is a useful indicator of CysDA-derived units in NM. The iron-binding characteristics of neuromelanin of the human substantia nigra were quantified and characterized by Double et al. (2003) who demonstrated that the iron-binding capacity of neuromelanin is 10-fold greater than that of a model synthetic melanin. This data was in agreement with the larger iron cluster size demonstrated by Mossbauer spectroscopy in the native pigment compared with the synthetic melanin. Faucheux et al. (2003) reported evidence that neuromelanin associated redox-active iron is increased (+69%) in the substantia nigra of patients with Parkinson's disease in accordance with the view that overloading of neuromelanin with redox-active elements may contribute to oxidative stress and intraneuronal damage in Parkinson's disease. Dzierzega-Lecznar et al. (2003) utilized pyrolysis/gas chromatography-mass spectrometry to investigate the effect of peroxynitrite, a potent oxidizing and nitrating agent derived from nitric oxide, on the structure of synthetic models of human neuromelanin. The results showed that interaction with peroxynitrite causes extensive oxidative degrdation of melanin pigments which may be of great biological importance in relation to depigmentation of nigrostriatal neurons in Parkinson's disease.

- Double Kay L., Gerlach Manfred, Schunemann Volker, Trautwein Alfred X, Zecca Luigi, Gallorini Mario, Youdim Moussa B. H., Riederer Peter, Ben-Shachar Dorit.
  Iron-binding characteristics of neuromelanin of the human substantia nigra. Biochemical Pharmacology 66(3):489-494, 2003.

- Double, K. L.; Halliday, G. M.; Henderson, J.; Griffiths, F. M.; Heinemann, T.; Riederer, P.; Gerlach, M.

The dopamine receptor agonist lisuride attenuates iron-mediated dopaminergic neurodegeneration. Experimental Neurology 184(1):530-535, 2003.

- Dzierzega-Lecznar, Anna; Stepien, Krystyna; Chodurek, Ewa; Kurkiewicz, Slawomir; Swiatkowska, Longina; Wilczok, Tadeusz.
  Pyrolysis-gas chromatography/mass spectrometry of peroxynitrite-treated melanins. Journal of Analytical and Applied Pyrolysis 70(2):457-467, 2003.

- Fahn, Stanley.
  Description of parkinson's disease as a clinical syndrome. Annals of the New York Academy of Sciences 991(Parkinson's Disease):1-14, 2003.

- Faucheux Baptiste A., Martin Marie-Elise, Beaumont Carole, Hauw Jean-Jacques, Agid Yves, Hirsch Etienne C.
  Neuromelanin associated redox-active iron is increased in the substantia nigra of patients with Parkinson's disease. Journal of Neurochemistry. 86(5) :1142-1148, 2003.

- Ide-Ektessabi Ari, Kawakami Takuo, Watt Frank.
  Distribution and chemical state analysis of iron in the Parkinsonian substantia nigra using synchrotron radiation micro beams. Nuclear Instruments & Methods in Physics Research, Section B: Beam Interactions with Materials and Atoms 213(Complete):590-594, 2004.

- Ito Shosuke, Wakamatsu Kazumasa, Zecca Luigi.
  Structure and function of neuromelanin. Advances in Behavioral Biology, 53(Catecholamine Research):269-272, 2002.

- Kawakami Takuo, ide-Ektessabi Ari, Watt Frank.
  Chemical state analysis of iron using synchrotron radiation micro beam - brain tissues of monkey with Parkinson's disease. Biomedical Research on Trace Elements 14(3): 210-214, 2003.

- Matsunaga Jun, Riley Patrick A., Solano Francisco, Hearing Vincent J.
  Biosynthesis of neuromelanin and melanin: the potential involvement of macrophage inhibitory factor and DOPAchrome tautomerase as rescue enzymes. Advances in Behavioral Biology, 53(Catecholamine Research):273-276, 2002.

- Wakamatsu Kazumasa, Fujikawa Kenichi, Zucca Fabio A.,; Zecca Luigi, Ito Shosuke.
  The structure of neuromelanin as studied by chemical degradative. Journal of Neurochemistry 86(4):1015-1023, 2003.

- Yoshida Sohei, Ektessabi Ari-Ide.
  Application of a synchrotron radiation micro beam : elemental and chemical state analyses at cellular level in Parkinson disease and amyotrophic lateral sclerosis. Biomedical Research on Trace Elements 4(3):196-203, 2003.

- Zecca Luigi, Zucca Fabio A., Wilms Henrik, Sulzer David.
  Neuromelanin of the substantia nigra: a neuronal black hole with protective and toxic characteristics. Trends in Neurosciences 26(11), 578-580, 2003.

- Zecca L., Zucca F. A., Costi P., Tampellini D., Gatti A., Gerlach M., Riederer P., Fariello R.G., Ito S., Gallorini M., Sulzer D.
  The neuromelanin of human substantia nigra: structure, synthesis and molecular behaviour. Advances in Research on Neurodegeneration 10:145-155, 2003.

6. Genetics, molecular and developmental biology

(Dr. F. Beermann)

Tyrosinase gene regulation: Three papers of the laboratories of Lluis Montoliu (**Regales et al., 2003; Giraldo et al., 2003**) and of Richard King (**Fryer et al., 2003**) address the upstream regulatory sequences of the tyrosinase gene. Both in human and mouse, several cis-regulatory elements reside within the first few hundred basepairs 5' to the gene, as for example the M-box, which is the binding site for the Microphthalmia transcription factor. In the mouse, at -15 kb, a further important region was detected more than 12 years ago, due to molecular analysis of mutant mice, which had a mottled pattern of

pigmentation (chinchilla-mottled mice, *Tyr$^{c\text{-}m}$*). By transfection and transgenic experiments, it was shown that this region has enhancing activity, and provides copy number-dependent and position-independent expression. **Giraldo et al**. have further analysed the mouse tyrosinase locus control region, and by using transgenic mice, transgenic flies (!) and transfection analyses, they now show that this region has boundary activity, protecting the tyrosinase gene regulation from negative effects of neighboring chromatin. Up to now, this sequence was not identified in any other vertebrate, which was mainly due to the unaccessibility of the complete genome sequence. Thus, it was only a matter of time until the human counterpart of this important region would be identified. **Fryer et al**. and **Regales et al**. have now compared the sequence of human tyrosinase upstream sequence to the -15 kb regulatory sequence in the mouse and have provided compellling evidence that the counterpart of the mouse tyrosinase locus control region has been identified in human tyrosinase and resides at -9kb 5' to the transcription start site. This site might even be a candidate region for yet unidentified mutations in patients with OCA type I.
(Part of the text above has been published in "In this issue... Pigment Cell Res 16: 605, 2003").

- Ancans J, Flanagan N, Hoogduijn MJ, Thody AJ.
P-locus is a target for the melanogenic effects of MC-1R signaling: a possible control point for facultative pigmentation. Ann N Y Acad Sci 994:373-377, 2003.

- Andersson L.
Melanocortin receptor variants with phenotypic effects in horse, pig, and chicken. Ann N Y Acad Sci 994:313-318, 2003.

- Botchkareva NV, Botchkarev VA, Gilchrest BA.
Fate of melanocytes during development of the hair follicle pigmentary unit. J Investig Dermatol Symp Proc 8(1):76-79, 2003.

- Chan KK, Wong CK, Lui VC, Tam PK, Sham MH.
Analysis of SOX10 mutations identified in Waardenburg-Hirschsprung patients: Differential effects on target gene regulation. J Cell Biochem 90(3):573-585, 2003.

- Cohen-Solal KA, Sood R, Marin Y, Crespo-Carbone SM, Sinsimer D, Martino JJ, Robbins C, Makalowska I, Trent J, Chen S.
Identification and characterization of mouse Rab32 by mRNA and protein expression analysis. Biochim Biophys Acta 1651(1-2):68-75, 2003.

- Delfgaauw J, Duschl J, Wellbrock C, Froschauer C, Schartl M, Altschmied J.
MITF-M plays an essential role in transcriptional activation and signal transduction in Xiphophorus melanoma. Gene 320:117-126, 2003.

- Du J, Miller AJ, Widlund HR, Horstmann MA, Ramaswamy S, Fisher DE.
MELANA/MART1 and SILV/PMEL17/GP100 are transcriptionally regulated by MITF in melanocytes and melanoma. Am J Pathol 163(1):333-343, 2003.

- Elworthy S, Lister JA, Carney TJ, Raible DW, Kelsh RN.
Transcriptional regulation of Mitfa accounts for the Sox10 requirement in zebrafish melanophore development. Development 130(12):2809-2818, 2003.
Summary: We propose that the dominant melanophore phenotype in Waardenburg syndrome IV individuals with SOX10 mutations is likely to result from failure to activate MITF in the normal number of melanoblasts.

- Filistowicz A, Przysiecki P, Wierzbicki H, Tokarska M.
Genetic parameters of coat colour in golden fox (Vulpes vulpes L.). J Appl Genet 41(4):259-265, 2000.

- Fryer J, Oetting W, King R.
Identification and characterization of a DNase hypersensitive region of the human tyrosinase gene. Pigment Cell Research 16:679-684, 2003. (See comment above)

- Gaggioli C, Busca R, Abbe P, Ortonne JP, Ballotti R.
Microphthalmia-associated transcription factor (MITF) is required but is not sufficient to induce the expression of melanogenic genes. Pigment Cell Res 16(4):374-382, 2003.

- Gimenez E, Lavado A, Giraldo P, Montoliu L.
Tyrosinase gene expression is not detected in mouse brain outside the retinal pigment epithelium cells. Eur J Neurosci 18(9):2673-2676, 2003.

- Giraldo P, Martinez A, Regales L, Lavado A, Garcia-Diaz A, Alonso A, Busturia A, Montoliu L.

Functional dissection of the mouse tyrosinase locus control region identifies a new putative boundary activity. Nucleic Acids Res 31(21):6290-6305., 2003.
Abstract: Locus control regions (LCRs) are complex high-order chromatin structures harbouring several regulatory elements, including enhancers and boundaries. We have analysed the mouse tyrosinase LCR functions, in vitro, in cell lines and, in vivo, in transgenic mice and flies. The LCR-core (2.1 kb), located at -15 kb and carrying a previously described tissue-specific DNase I hypersensitive site, operates as a transcriptional enhancer that efficiently transactivates heterologous promoters in a cell-specific orientation-independent manner. Furthermore, we have investigated the boundary activity of these sequences in transgenic animals and cells. In mice, the LCR fragment (3.7 kb) rescued a weakly expressed reference construct that displays position effects. In Drosophila, the LCR fragment and its core insulated the expression of a white minigene reporter construct from chromosomal position effects. In cells, sequences located 5' from the LCR-core displayed putative boundary activities. We have obtained genomic sequences surrounding the LCR fragment and found a LINE1 repeated element at 5'. In B16 melanoma and L929 fibroblast mouse cells, this element was found heavily methylated, supporting the existence of putative boundary elements that could prevent the spreading of condensed chromatin from the LINE1 sequences into the LCR fragment, experimentally shown to be in an open chromatin structure.

- Graw J, Pretsch W, Loster J.
Mutation in intron 6 of the hamster Mitf gene leads to skipping of the subsequent exon and creates a novel animal model for the human Waardenburg syndrome type II. Genetics 164(3):1035-1041, 2003.
Shortened abstract: In the course of analysis of ENU-induced mutations in Syrian hamsters, a novel dominant anophthalmic white mutant (Wh(V203)) with hearing loss was recovered.
The Wh(V203) mutation in the Syrian hamster affects the same functional domains of the Mitf transcription factor as the human R124X mutation, causing human Waardenburg syndrome type II. Therefore, the Wh(V203) hamster mutant provides a novel model for this particular syndrome.

- He L, Eldridge AG, Jackson PK, Gunn TM, Barsh GS.
Accessory proteins for melanocortin signaling: attractin and mahogunin. Ann N Y Acad Sci 994:288-298, 2003.

- Huber WE, Price ER, Widlund HR, Du J, Davis IJ, Wegner M, Fisher DE.
A tissue-restricted cAMP transcriptional response: SOX10 modulates alpha-melanocyte-stimulating hormone-triggered expression of microphthalmia-associated transcription factor in melanocytes. J Biol Chem 278(46):45224-45230, 2003.

- King RA, Pietsch J, Fryer JP, Savage S, Brott MJ, Russell-Eggitt I, Summers CG, Oetting WS.
Tyrosinase gene mutations in oculocutaneous albinism 1 (OCA1): definition of the phenotype. Hum Genet 113(6):502-513., 2003.

- Klungland H, Vage DI.
Pigmentary switches in domestic animal species. Ann N Y Acad Sci 994:331-338., 2003.

- Knight RD, Nair S, Nelson SS, Afshar A, Javidan Y, Geisler R, Rauch GJ, Schilling TF.
lockjaw encodes a zebrafish tfap2a required for early neural crest development. Development 130(23):5755-5768, 2003.
Shortened abstract: The neural crest is a uniquely vertebrate cell type that gives rise to much of the craniofacial skeleton, pigment cells and peripheral nervous system, yet its specification and diversification during embryogenesis are poorly understood. Zebrafish homozygous for the lockjaw (low) mutation show defects in all of these derivatives and we show that low (allelic with montblanc) encodes a zebrafish tfap2a, one of a small family of transcription factors implicated in epidermal and neural crest development.
These studies demonstrate that low is required for early steps in neural crest development and suggest that tfap2a is essential for the survival of a subset of neural crest derivatives.

- Kumasaka M, Sato S, Yajima I, Yamamoto H.
Isolation and developmental expression of tyrosinase family genes in Xenopus laevis. Pigment Cell Res 16(5):455-462, 2003.

- Majerus ME, Mundy NI.
Mammalian melanism: natural selection in black and white. Trends Genet 19(11):585-588, 2003.
Abstract: Two recent papers on the molecular basis of melanism strengthen the chain of evidence linking genotype and phenotype in nature. Research on coat colour polymorphisms in rock pocket mice from differently coloured rock substrates provides a compelling example of the genetics of adaptation and the serendipitous nature of darwinian selection. Mutations in one gene, melanocortin-1-receptor, are perfectly associated with dark coat colour on black lava. Comparative sequence analysis shows that the same gene is involved in melanic polymorphism in some cats.

- McKenzie CA, Harding RM, Tomlinson JB, Ray AJ, Wakamatsu K, Rees JL.

Phenotypic expression of melanocortin-1 receptor mutations in Black Jamaicans. J Invest Dermatol 121(1):207-208, 2003.

- Mundy NI, Kelly J, Theron E, Hawkins K.
Evolutionary genetics of the melanocortin-1 receptor in vertebrates. Ann N Y Acad Sci 994:307-312, 2003.

- Naraoka T, Uchisawa H, Mori H, Matsue H, Chiba S, Kimura A.
Purification, characterization and molecular cloning of tyrosinase from the cephalopod mollusk, Illex argentinus. Eur J Biochem 270(19):4026-4038, 2003.

- Nazarian R, Falcon-Perez JM, Dell'Angelica EC.
Biogenesis of lysosome-related organelles complex 3 (BLOC-3): a complex containing the Hermansky-Pudlak syndrome (HPS) proteins HPS1 and HPS4. Proc Natl Acad Sci U S A 100(15):8770-8775, 2003.

- O'Hagan RC, Brennan CW, Strahs A, Zhang X, Kannan K, Donovan M, Cauwels C, Sharpless NE, Wong WH, Chin L.
Array comparative genome hybridization for tumor classification and gene discovery in mouse models of malignant melanoma. Cancer Res 63(17):5352-5356, 2003.

- Rawls JF, Johnson SL.
Temporal and molecular separation of the kit receptor tyrosine kinase's roles in zebrafish melanocyte migration and survival. Dev Biol 262(1):152-161., 2003.

- Rees JL.
Genetics of hair and skin color. Annu Rev Genet 37:67-90., 2003.

- Regales L, Giraldo P, Garcia-Diaz A, Lavado A, Montoliu L.
Identification and functional validation of a 5' upstream regulatory sequence in the human tyrosinase gene homologous to the locus control region of the mouse tyrosinase gene. Pigment Cell Research 16:685-692, 2003.
(see comment above)

- Rosenwald IB, Wang S, Savas L, Woda B, Pullman J.
Expression of translation initiation factor eIF-2alpha is increased in benign and malignant melanocytic and colonic epithelial neoplasms. Cancer 98(5):1080-1088., 2003.

- Rouzaud F, Annereau JP, Valencia JC, Costin GE, Hearing VJ.
Regulation of melanocortin 1 receptor expression at the mRNA and protein levels by its natural agonist and antagonist. Faseb J 17(14):2154-2156., 2003.

- Smart JL, Low MJ.
Lack of proopiomelanocortin peptides results in obesity and defective adrenal function but normal melanocyte pigmentation in the murine C57BL/6 genetic background. Ann N Y Acad Sci 994:202-210., 2003.

- Sturm RA, Duffy DL, Box NF, Newton RA, Shepherd AG, Chen W, Marks LH, Leonard JH, Martin NG.
Genetic association and cellular function of MC1R variant alleles in human pigmentation. Ann N Y Acad Sci 994:348-358., 2003.

- Suto J, Sekikawa K.
Genetic determinants of sable and umbrous coat color phenotypes in mice. Pigment Cell Res 16(4):388-396., 2003.

- Suzuki N, Hirata M, Kondo S.
Traveling stripes on the skin of a mutant mouse. Proc Natl Acad Sci U S A 100(17):9680-9685., 2003.
Abstract: In the course of animal development, complex structures form autonomously from the apparently shapeless egg. How cells can produce spatial patterns that are much larger than each cell is one of the key issues in developmental biology. It has been suggested that spatial patterns in animals form through the same principles by which dispatched structures are formed in the nonbiological system. However, because of the complexity of biological systems, molecular details of such phenomena have been rarely clarified. In this article, we introduce an example of a pattern-forming phenomenon that occurs in the skin of mutant mice. The mutant mouse has a defect in splicing of the Foxn1 (Whn or nude) gene, which terminates hair follicle development just after pigment begins to accumulate in the follicle. The immature follicles are rapidly discharged, and a new hair cycle resumes. Eventually, the skin color of the mouse appears to oscillate. The color oscillation is synchronous in juvenile mice, but the phase gradually shifts among skin regions to eventually form traveling, evenly spaced stripes. Although the time scale is quite different, the pattern change in the mutant mouse shares characteristics with the nonlinear waves generated on excitable media, such as the Belousov-Zhabotinskii reaction, suggesting that a common principle underlies the wave pattern formation. Molecular details that underlie the phenomenon can be conjectured from recent molecular studies.

- Tachibana M, Kobayashi Y, Matsushima Y.
Mouse models for four types of Waardenburg syndrome. Pigment Cell Res 16(5):448-454., 2003.

- Tonks ID, Nurcombe V, Paterson C, Zournazi A, Prather C, Mould AW, Kay GF.
Tyrosinase-Cre mice for tissue-specific gene ablation in neural crest and neuroepithelial-derived tissues. Genesis 37(3):131-138, 2003.
See for comparison: Tyr-Cre (Delmas et al., Genesis 36: 73-80, 2003), Tyrp1-Cre (Mori et al., Invest Ophthalmol Vis Sci: 43, 1384-1388), Dct-Cre (Guyonneau et al., Pigment Cell Res: 15, 305-309, 2002).

- Zuidervaart W, Van Der Velden PA, Hurks MH, Van Nieuwpoort FA, Out-Luiting CJ, Singh AD, Frants RR, Jager MJ, Gruis NA.
Gene expression profiling identifies tumour markers potentially playing a role in uveal melanoma development. Br J Cancer 89(10):1914-1919., 2003.

7. Tyrosinase, TRPs, other enzymes

(Prof. J.C. Garcia-Borron)

- Berson JF, Theos AC, Harper DC, Tenza D, Raposo G, Marks MS.
Proprotein convertase cleavage liberates a fibrillogenic fragment of a resident glycoprotein to initiate melanosome biogenesis. J Cell Biol. 161(3):521-33, 2003.
Lysosome-related organelles are cell type-specific intracellular compartments with distinct morphologies and functions. The molecular mechanisms governing the formation of their unique structural features are not known. Melanosomes and their precursors are lysosome-related organelles that are characterized morphologically by intralumenal fibrous striations upon which melanins are polymerized. The integral membrane protein Pmel17 is a component of the fibrils and can nucleate their formation in the absence of other pigment cell-specific proteins. Here, we show that formation of intralumenal fibrils requires cleavage of Pmel17 by a furin-like proprotein convertase (PC). As in the generation of amyloid, proper cleavage of Pmel17 liberates a lumenal domain fragment that becomes incorporated into the fibrils; longer Pmel17 fragments generated in the absence of PC activity are unable to form organized fibrils. Our results demonstrate that PC-dependent cleavage regulates melanosome biogenesis by controlling the fibrillogenic activity of a resident protein. Like the pathologic process of amyloidogenesis, the formation of other tissue-specific organelle structures may be similarly dependent on proteolytic activation of physiological fibrillogenic substrates.

- Cohen-Solal KA, Sood R, Marin Y, Crespo-Carbone SM, Sinsimer D, Martino JJ, Robbins C, Makalowska I, Trent J, Chen S.
Identification and characterization of mouse Rab32 by mRNA and protein expression analysis.
Biochim Biophys Acta. 1651(1-2):68-75, 2003.
Rab proteins, a subfamily of the ras superfamily, are low molecular weight GTPases involved in the regulation of intracellular vesicular transport. Cloning of human RAB32 was recently described. Presently, we report the cloning and characterization of the mouse homologue of Rab32. We show that murine Rab32 exhibits a ubiquitous expression pattern, with tissue-specific variation in expression level. Three cell types with highly specialized organelles, melanocytes, platelets and mast cells, exhibit relatively high level of Rab32. We show that in murine amelanotic in vitro transformed melanocytes as well as in human amelanotic metastatic melanoma cell lines, the expression of Rab32 is markedly reduced or absent, in parallel with the loss of expression of two key enzymes for the production of melanin, tyrosinase and Tyrp1. Therefore, in both mouse and human systems, the expression of Rab32 correlates with the expression of genes involved in pigment production. However, in melanoma samples, amelanotic due to a mutation in the tyrosinase gene, the expression of Rab32 remains at levels comparable to those observed in pigmented melanoma samples. Finally, we observed co-localization of Rab32 and the melanosomal proteins, Tyrp1 and Dct, indicating an association of Rab32 with melanosomes. Based on these data, we propose the inclusion of Rab32 to the so-called melanocyte/platelet family of Rab proteins.

- Di Donato P, Napolitano A
1,4-benzothiazines as key intermediates in the biosynthesis of red hair pigment pheomelanins. Pigment Cell Res. 16(5):532-539, 2003.
Following the discovery of cysteinyldopas as the early intermediates in the biogenesis of pheomelanins, the typical red hair pigments, the reactivity of the biosynthetic precursors under biomimetic conditions was extensively investigated. As a result, the early stages of pheomelanogenesis were envisaged as involving oxidative cyclization of cysteinyldopas, mainly the 5-S-isomer, to 1,4-benzothiazine (BTZ) intermediates which undergo oxidative polymerisation leading eventually to the pigments. In the last decade, several aspects of the chemistry and biosynthesis of pheomelanins were re-examined. In particular, (i) transient BTZ intermediates were identified by pulse radiolytic techniques and NMR analysis; (ii) the effect of reaction conditions and additives on the rearrangement vs. redox

exchange reaction paths of such intermediates were investigated in detail; (iii) the mechanism of the oxidative polymerization of BTZs was characterized by the first isolation of oligomer species, and (iv) the pigment eventually resulting from oxidation of 5-S-cysteinyldopa (CD) was directly analyzed by spectroscopic and chemical methodologies in comparison with pheomelanins isolated from human hair. These advances led eventually to an integrated picture of the biogenetic route highlighting the intervention of various chemical and enzymatic factors which affect the kinetics of the different steps and the nature of the key benzothiazine precursors. A likely biogenetic route was also postulated for the delta2,2'-bi(2H-1,4-benzothiazine) pigments, termed trichochromes, whose origin had remained an open issue since their first isolation from red human hair and avian feathers. Finally, a more detailed description of the structure of pheomelanin pigments in terms of the monomer units, their mode of linking, and postsynthetic modifications was gained.

- Du J, Miller AJ, Widlund HR, Horstmann MA, Ramaswamy S, Fisher DE.
MLANA/MART1 and SILV/PMEL17/GP100 are transcriptionally regulated by MITF in melanocytes and melanoma. Am J Pathol. 163(1):333-43, 2003.
The clinically important melanoma diagnostic antibodies HMB-45, melan-A, and MITF (D5) recognize gene products of the melanocyte-lineage genes SILV/PMEL17/GP100, MLANA/MART1, and MITF, respectively. MITF encodes a transcription factor that is essential for normal melanocyte development and appears to regulate expression of several pigmentation genes. In this report, the possibility was examined that MITF might additionally regulate expression of the SILV and MLANA genes. Both genes contain conserved MITF consensus DNA sequences that were bound by MITF in vitro and in vivo, based on electrophoretic mobility shift assay and chromatin-immunoprecipitation. In addition, MITF regulated their promoter/enhancer regions in reporter assays, and up- or down-regulation of MITF produced corresponding modulation of endogenous SILV and MLANA in melanoma cells. Expression patterns were compared with these factors in a series of melanoma cell lines whose mutational status of the proto-oncogene BRAF was also known. SILV and MLANA expression correlated with MITF, while no clear correlation was seen relative to BRAF mutation. Finally, mRNA expression array analysis of primary human melanomas demonstrated a tight correlation in their expression levels in clinical tumor specimens. Collectively, this study links three important melanoma antigens into a common transcriptional pathway regulated by MITF.

- Gaggioli C, Busca R, Abbe P, Ortonne JP, Ballotti R.
Microphthalmia-associated transcription factor (MITF) is required but is not sufficient to induce the expression of melanogenic genes. Pigment Cell Res. 16(4):374-82, 2003.
Microphthalmia-associated transcription factor (MITF) plays a pivotal role in melanocyte survival and differentiation. Nevertheless, until now it has not been possible to show that MITF regulates the expression of the endogenous tyrosinase or Tyrp1. Further, a direct involvement of MITF in the regulation of melanin synthesis, a key parameter of melanocyte differentiation, remains to be demonstrated. In the present report, using recombinant adenovirus encoding the wild-type or a dominant negative form of MITF, as well as stable cell lines expressing tetracycline inducible wild-type MITF, we reassessed the role of MITF in melanocyte differentiation and in the regulation of melanin synthesis. Immunofluorescence studies, as well as Western blot analyses, show that infection of B16 mouse melanoma cells or human melanocytes with adenovirus encoding wild-type MITF does not increase the expression of the endogenous melanogenic enzymes. However, infection with the MITF dominant negative mutant inhibits the expression of endogenous tyrosinase and Tyrp1 proteins and blocks cAMP-induced melanin synthesis. Thus, MITF is required but does not seem to be sufficient to induce the expression of melanogenic enzymes and we show for the first time a direct involvement of MITF in the regulation of melanin pigment synthesis. As a whole, our data point to the existence of still unknown regulatory mechanisms that co-operate or synergize with MITF to control melanogenic gene expression and melanin synthesis. The identification of such mechanisms will greatly improve our understanding of the melanocyte differentiation processes.

- Giraldo P, Martinez A, Regales L, Lavado A, Garcia-Diaz A, Alonso A, Busturia A, Montoliu L.
Functional dissection of the mouse tyrosinase locus control region identifies a new putative boundary activity. Nucleic Acids Res. 31(21):6290-305, 2003.
Locus control regions (LCRs) are complex high-order chromatin structures harbouring several regulatory elements, including enhancers and boundaries. We have analysed the mouse tyrosinase LCR functions, in vitro, in cell lines and, in vivo, in transgenic mice and flies. The LCR-core (2.1 kb), located at -15 kb and carrying a previously described tissue-specific DNase I hypersensitive site, operates as a transcriptional enhancer that efficiently transactivates heterologous promoters in a cell-specific orientation-independent manner. Furthermore, we have investigated the boundary activity of these sequences in transgenic animals and cells. In mice, the LCR fragment (3.7 kb) rescued a weakly expressed reference construct that displays position effects. In Drosophila, the LCR fragment and its core insulated the expression of a white minigene reporter construct from chromosomal position effects. In cells, sequences located 5' from the LCR-core displayed putative boundary activities. We have obtained genomic sequences surrounding the LCR fragment and found a LINE1 repeated element at 5'. In B16 melanoma and L929 fibroblast mouse cells, this element was found heavily methylated, supporting the existence of putative boundary elements that could prevent the spreading of condensed chromatin from the LINE1 sequences into the LCR fragment, experimentally shown to be in an open chromatin structure.

- Hasegawa T, Matsuzaki M, Takeda A, Kikuchi A, Furukawa K, Shibahara S, Itoyama Y.
  Increased dopamine and its metabolites in SH-SY5Y neuroblastoma cells that express tyrosinase. J Neurochem. 87(2):470-5, 2003.
  Oxidized metabolites of dopamine, known as dopamine quinone derivatives, are thought to play a pivotal role in the degeneration of dopaminergic neurons. Although such quinone derivatives are usually produced via the autoxidation of catecholamines, tyrosinase, which is a key enzyme in melanin biosynthesis via the production of DOPA and subsequent molecules, may potentially accelerate the induction of catecholamine quinone derivatives by its oxidase activity. In the present study, we developed neuronal cell lines in which the expression of human tyrosinase was inducible. Overexpression of tyrosinase in cultured cell lines resulted in (i) increased intracellular dopamine content; (ii) induction of oxidase activity not only for DOPA but also for dopamine; (iii) formation of melanin pigments in cell soma; and (iv) increased intracellular reactive oxygen species. Interestingly, the expressed tyrosinase protein was initially distributed in the entire cytoplasm and then accumulated to form catecholamine-positive granular structures by 3 days after the induction. The granular structures consisted of numerous rounded, dark bodies of melanin pigments and were largely coincident with the distribution of lysosomes. This cellular model that exhibits increased dopamine production will provide a useful tool for detailed analyses of the potentially noxious effects of oxidized catecholamine metabolites.

- Ito S, Wakamatsu K.
  Quantitative analysis of eumelanin and pheomelanin in humans, mice, and other animals: a comparative review. Pigment Cell Res. 16(5):523-31, 2003.
  The color of hair, skin, and eyes in animals mainly depends on the quantity, quality, and distribution of the pigment melanin, which occurs in two types: black to brown eumelanin and yellow to reddish pheomelanin. Microanalytical methods to quantify the amounts of eumelanin and pheomelanin in biological materials were developed in 1985. The methods are based on the chemical degradation of eumelanin to pyrrole-2,3,5-tricarboxylic acid and of pheomelanin to aminohydroxyphenylalanine isomers, which can be analyzed and quantitated by high performance liquid chromatography. This review summarizes and compares eumelanin and pheomelanin contents in various pigmented tissues obtained from humans, mice, and other animals. These methods have become valuable tools to study the functions of melanin, the control of melanogenesis, and the actions and interactions of pigmentation genes. The methods have also found applications in many clinical studies. High levels of pheomelanin are found only in yellow to red hairs of mammals and in red feathers of birds. It remains an intriguing question why lower vertebrates such as fishes do not synthesize pheomelanin. Detectable levels of pheomelanin are detected in human skin regardless of race, color, and skin type. However, eumelanin is always the major constituent of epidermal melanin, and the skin color appears to be determined by the quantity of melanin produced but not by the quality.

- Kadekaro AL, Kavanagh RJ, Wakamatsu K, Ito S, Pipitone MA, Abdel-Malek ZA.
  Cutaneous photobiology. The melanocyte vs. the sun: who will win the final round? Pigment Cell Res. 16(5):434-47, 2003.
  Solar ultraviolet radiation (UV) is a major environmental factor that dramatically alters the homeostasis of the skin as an organ by affecting the survival, proliferation and differentiation of various cutaneous cell types. The effects of UV on the skin include direct damage to DNA, apoptosis, growth arrest, and stimulation of melanogenesis. Long-term effects of UV include photoaging and photocarcinogenesis. Epidermal melanocytes synthesize two main types of melanin: eumelanin and pheomelanin. Melanin, particularly eumelanin, represents the major photoprotective mechanism in the skin. Melanin limits the extent of UV penetration through the epidermal layers, and scavenges reactive oxygen radicals that may lead to oxidative DNA damage. The extent of UV-induced DNA damage and the incidence of skin cancer are inversely correlated with total melanin content of the skin. Given the importance of the melanocyte in guarding against the adverse effects of UV and the fact that the melanocyte has a low self-renewal capacity, it is critical to maintain its survival and genomic integrity in order to prevent malignant transformation to melanoma, the most fatal form of skin cancer. Melanocyte transformation to melanoma involves the activation of certain oncogenes and the inactivation of specific tumor suppressor genes. This review summarizes the current state of knowledge about the role of melanin and the melanocyte in photoprotection, the responses of melanocytes to UV, the signaling pathways that mediate the biological effects of UV on melanocytes, and the most common genetic alterations that lead to melanoma.

- Kim DS, Park SH, Kwon SB, Joo YH, Youn SW, Sohn UD, Park KC.
  Temperature regulates melanin synthesis in melanocytes. Arch Pharm Res. 26(10):840-5, 2003.
  Temperature change is one of the major environmental factors that influence the human skin. However, the relationship between temperature and melanogenesis has received little attention. In the present study, we investigated the effects of temperature change on melanogenesis in a mouse melanocyte cell line (Mel-Ab), and primary cultured human melanocytes. We found that Mel-Ab cells cultured at low temperatures (31 and 34 degrees C) produce less melanin than cells at 37 degrees C. These results were confirmed by experiments upon human melanocytes, demonstrating that the hypopigmenting effect of low temperatures is not cell type dependent. The observed melanin production was found to be accompanied by tyrosinase activity at each temperature, indicating that tyrosinase activity is regulated by temperature. We further examined whether the incubation period at low temperatures plays an important role in the regulation of melanogenesis. Short exposures to 27 degrees C for 1 h or 3 h did not affect

tyrosinase activity or melanin synthesis, whereas long exposures to 31 degrees C for 2 days or 6 days significantly reduced tyrosinase activity and melanin synthesis in a duration-dependent manner. Our results suggest that exposure to low temperature and the duration of this exposure are important regulators of melanogenesis.

- Kim TJ, Cho MK, Lee JS, Whang KU, Jin SY, Hoshino T.
The expression of melanogenic proteins in Korean skin after ultraviolet irradiation. J Dermatol. 2003 Sep;30(9):665-72.
For proper melanin production, several specific enzymes such as tyrosinase, tyrosinase-related protein 1 (TRP-1) and dopachrome tautomerase are required. Their expressions are increased after exposure to UVB. However, it is not known how long tyrosinase and TRP-1 activities continue after UV irradiation in vivo. The purpose of this study is to measure the changes in expressions of tyrosinase, TRP1, and MITF after exposure to UV on skin in a Korean population. We established an immunohistochemical staining protocol for specimens which were obtained from UV-irradiated skin in five healthy Korean males on the 2nd, 5th, 7th, 28th, and 56th days after UV irradiation. Tyrosinase, TRP-1, and MITF expressions increased until 7 days after UV irradiation and then dropped to the basal constitutive level 4 and 8 weeks later. Interestingly, tyrosinase increased prior to TRP-1. This study reveals the time-sequence of melanin-synthesized enzymes and provides important information for the clinical evaluation of the effectiveness of whitening agents.

- King RA, Pietsch J, Fryer JP, Savage S, Brott MJ, Russell-Eggitt I, Summers CG,Oetting WS.
Tyrosinase gene mutations in oculocutaneous albinism 1 (OCA1): definition of the phenotype. Hum Genet. 113(6):502-13, 2003.
Oculocutaneous albinism (OCA) is a common human genetic condition resulting from mutations in at least twelve different genes. OCA1 results from mutations of the tyrosinase gene and presents with the life-long absence of melanin pigment after birth (OCA1A) or with the development of minimal-to-moderate amounts of cutaneous and ocular pigment (OCA1B). Other types of OCA have variable amounts of cutaneous and ocular pigment. We hypothesized that white hair at birth indicates OCA1 and tested this in a sample of 120 probands with OCA and white hair at birth. We found that 102 (85%) of the probands had OCA1 with one or two identifiable tyrosinase gene mutations, with 169 (83%) of the 204 OCA1 tyrosinase gene alleles having identifiable mutations and 35 (17%) having no identifiable change in the coding, splice junction, or proximal promoter regions of the gene. The inability to identify the mutation was more common with OCA1B (24/35, 69%) than with OCA1A (11/35, 31%) alleles. Seven probands with no tyrosinase gene mutations were found to have OCA2 with one or two P gene mutations, and in eleven, no mutations were detected in either gene. We conclude that (1) the presence of white hair at birth is a useful clinical tool suggesting OCA1 in a child or adult with OCA, although OCA2 may also have this presentation; (2) the molecular analysis of the tyrosinase and P genes are necessary for precise diagnosis; and (3) the presence of alleles without identifiable mutations of the tyrosinase gene, particularly in OCA1B, suggests that more complex mutation mechanisms of this gene are common in OCA.

- Kumasaka M, Sato S, Yajima I, Yamamoto H.
Isolation and developmental expression of tyrosinase family genes in Xenopus laevis. Pigment Cell Res. 16(5):455-62, 2003.
The tyrosinase family of genes in vertebrates consists of three related members encoding melanogenic enzymes, tyrosinase (Tyr), tyrosinase-related protein-1 (TRP-1, Tyrp1) and tyrosinase-related protein-2 (Dct, TRP-2, Tyrp2). These proteins catalyze melanin production in pigment cells and play important roles in determining vertebrate coloration. This is the first report examining melanogenic gene expression in pigment cells during embryonic development of amphibians. Xenopus provides a useful experimental system for analysing molecular mechanisms of pigment cells. However, in this animal little information is available not only about the developmental expression but also about the isolation of pigmentation genes. In this study, we isolated homologues of Tyr, Tyrp1 and Dct in Xenopus laevis (XlTyr, XlTyrp1, and XlDct). We studied their expression during development using in situ hybridization and found that all of them are expressed in neural crest-derived melanophores, most of which migrate through the medial pathway, and in the developing diencephalon-derived retinal pigment epithelium (RPE). Further, XlDct was expressed earlier than XlTyr and XlTyrp1, which suggests that XlDct is the most suitable marker gene for melanin-producing cells among them. XlDct expression was detected in migratory melanoblasts and in the unpigmented RPE. In addition, the expression of XlDct was detected in the pineal organ. The sum of these studies suggests that expression of the tyrosinase family of genes is conserved in pigment cells of amphibians and that using XlDct as a marker gene for pigment cells will allow further study of the developmental mechanisms of pigment cell differentiationusing Xenopus.

- Land EJ, Ito S, Wakamatsu K, Riley PA.
Rate constants for the first two chemical steps of eumelanogenesis. Pigment Cell Res 16(5):487-493, 2003.

- Land EJ, Ramsden CA, Riley PA, Yoganathan G.
Mechanistic studies of catechol generation from secondary quinone amines relevant to indole formation and tyrosinase activation. Pigment Cell Res. 16(4):397-406, 2003.

The biological significance of the spontaneous cyclization and redox reactions of ortho-quinone amines is that these appear to be the mechanism of formation of the indolic components of melanin and are also involved in the autoactivation of tyrosinase. We have previously shown that activation of tyrosinase is prevented by the formation of a cyclic betaine from a tertiary amine analogue. Evidence is presented to show that cyclization of ortho-quinones by Michael addition also occurs in the oxidation of secondary catecholamines. Three varieties of cyclic product have been detected and their formation is influenced by the nature of the N-substituent. Five-membered betaine rings form directly and, although six- and seven-membered rings also form, a transient spiro isomer of the ortho-quinone was in some cases detected as an intermediate. The heterocyclic products formed as betaines undergo redox exchange with residual quinone to form the corresponding aminochromes. We have established the kinetic constants of these reactions, either directly by pulse radiolysis measurements or by inference using a computer model of the reaction pathway to fit the observed data. To investigate the potential biological applications of this chemistry the system was also examined by tyrosinase-catalysed oxidation of the catecholamine substrates in which there is re-oxidation of the catechol formed by the redox exchange reaction and enables measurement of oxygen utilization stoichiometry. We show that the redox exchange reaction is unaffected by side-chain modification whereas cyclization is dependent on both electronic and steric factors. In the light of these studies we conclude that the failure of tertiary amine-derived betaines to undergo redox exchange, and thus block in vitro activation of tyrosinase, is due to the absence of a second exchangeable proton.

- Lee JY, Kang WH.
Effect of cyclosporin A on melanogenesis in cultured human melanocytes. Pigment Cell Res16(5):504-8, 2003.
Cyclosporin A (CsA) is a widely used immunosuppressant. Reports on the effect of CsA on hyperpigmentation in patients appear inconsistent, and the effect of CsA on skin pigment cells (melanocytes) in vitro is unknown. We examined the effect of CsA on human melanocyte proliferation and melanogenesis in vitro. Melanocyte proliferation was dose-dependently inhibited by 0.1-10 microM CsA, with no effect on cell viability. Melanocytes incubated with 10 microM CsA for 6 days showed decreased pigmentation and tyrosinase activity. Western blot analysis using an anti-tyrosinase antibody revealed that CsA (0.1-10 microM) decreased tyrosinase protein levels in a dose-dependent manner. Northern blot analysis showed similar effects on tyrosinase mRNA levels. These effects of CsA on melanogenesis in vitro are not consistent with suggestions that systemic CsA therapy causes patient skin hyperpigmentation.

- Nakamura K, Yoshida M, Uchiwa H, Kawa Y, Mizoguchi M.
Down-regulation of melanin synthesis by a biphenyl derivative and its mechanism. Pigment Cell Res. 16(5):494-500, 2003.
Down-regulation of melanin synthesis is required for recovery of pigmentary disorders and it is known that direct inhibitors of tyrosinase, the key enzyme in melanin synthesis, such as hydroquinone with a phenol structure, suppress melanin synthesis. We screened several phenolic derivatives using B16 melanoma cells and found that a biphenyl derivative, 2,2'-dihydroxy-5,5'-dipropyl-biphenyl (DDB), down-regulated melanin synthesis effectively. Although DDB has a phenol structure, it did not inhibit tyrosinase in vitro, thus we examined its mechanism in detail. Western blotting revealed that the amount of tyrosinase was decreased by DDB, and pulse-chase labeling and immunoprecipitation analysis showed a decrease of mature tyrosinase and acceleration of tyrosinase degradation in its presence. These results suggest that DDB down-regulates melanin synthesis by inhibiting the maturation of tyrosinase, leading to acceleration of tyrosinase degradation.

- Naraoka T, Uchisawa H, Mori H, Matsue H, Chiba S, Kimura A.
Purification, characterization and molecular cloning of tyrosinase from the cephalopod mollusk, Illex argentinus. Eur J Biochem. 270(19):4026-38, 2003.
Tyrosinase (monophenol, L-DOPA:oxygen oxidoreductase) was isolated from the ink of the squid, Illex argentinus. Squid tyrosinase, termed ST94, was found to occur as a covalently linked homodimeric protein with a molecular mass of 140.2 kDa containing two copper atoms per a subunit. The tyrosinase activity of ST94 was enhanced by proteolysis with trypsin to form a protein, termed ST94t, with a molecular mass of 127.6 kDa. The amino acid sequence of the subunit was deduced from N-terminal amino acid sequencing and cDNA cloning, indicating that the subunit of ST94 is synthesized as a premature protein with 625 amino acid residues and an 18-residue signal sequence region is eliminated to form the mature subunit comprised of 607 amino acid residues with a deduced molecular mass of 68,993 Da. ST94 was revealed to contain two putative copper-binding sites per a subunit, that showed sequence similarities with those of hemocyanins from mollusks, tyrosinases from microorganisms and vertebrates and the hypothetical tyrosinase-related protein of Caenorhabditis elegans. The squid tyrosinase was shown to catalyze the oxidation of monophenols as well as o-diphenols and to exhibit temperature-dependency of o-diphenolase activity like a psychrophilic enzyme.

- Ohguchi K, Banno Y, Akao Y, Nozawa Y.
Involvement of phospholipase D1 in melanogenesis of mouse B16 melanoma cells. J Biol Chem. 2003.
In response to alpha-melanocyte-stimulating hormone (alpha-MSH) or cAMP-elevating agents, mouse B16 melanoma cells underwent differentiation characterized by increased melanin biosynthesis. However, the mechanism(s) underlying the regulation of melanogenesis during differentiation has not yet been clearly understood. Phospholipase D

(PLD) has been reported to be involved in differentiation. This enzyme cleaves phosphatidylcholine upon stimulation with stimuli to generate phosphatidic acid. In the current study, the involvement of PLD in the regulation of melanogenesis characteristic of differentiation was examined using mouse B16 melanoma cells. Treatment of B16 cells with alpha-MSH was found to cause marked decreases in the PLD1 activity concurrent with its reduced protein level. Moreover, treatment of exogenous bacterial PLD also inhibited alpha-MSH-induced melanogenesis. To further investigate the role of PLD1 in the regulation of melanogenesis, we examined the effects of overexpression of PLD1 on melanogenesis in B16 melanoma cells. The B16 cells overexpressing PLD were prepared by transfection with the vector containing the cDNA encoding PLD1. The melanin contents in PLD1-overexpressing cells (B16/PLD1) were observed to be lower compared with those in the vector control cells (B16/Vec), concomitant with the decreases in both activity and protein level of tyrosinase, a key regulatory enzyme in melanogenesis. Moreover, overexpression of PLD1 resulted in a marked inhibition of melanogenesis induced by alpha-MSH. The inhibition of melanogenesis was well correlated with the decrease in the tyrosinase activity associated with its expression. These results indicated that PLD1 negatively regulated the melanogenic signaling bymodulating the expression of tyrosinase in mouse B16 melanoma cells.

- Ohguchi K, Tanaka T, Kido T, Baba K, Iinuma M, Matsumoto K, Akao Y, Nozawa Y.
Effects of hydroxystilbene derivatives on tyrosinase activity. Biochem Biophys Res Commun. 307(4):861-3, 2003.
Synthesis of melanin starts from the conversion of L-tyrosine to 3,4-dihydroxyphenylalanine (L-dopa) and then the oxidation of L-dopa yields dopaquinone by tyrosinase. Therefore, tyrosinase inhibitors have been established as important constituents of depigmentation agents. Recently, polyhydroxystilbene compounds, which are trans-resveratrol (3,4(')5-trihydroxy-trans-stilbene) analogs, have been demonstrated as potent tyrosinase inhibitors. However, their detailed inhibitory mechanisms are not clearly understood. In the present study, a variety of synthesized hydroxystilbene compounds were tested for their inhibitory effects against murine tyrosinase activity. The inhibitory potencies of the hydroxy-trans-stilbene compounds were remarkably elevated by increasing number of phenolic hydroxy substituents. Methylated hydroxy-trans-stilbene lost the inhibitory activity. Furthermore, hydrogenated hydroxystilbene or hydroxy-cis-stilbene exerted little or no inhibitory effect compared with hydroxy-trans-stilbene on tyrosinase activity. The structure-activity relationships demonstrated in the present study suggest that the phenolic hydroxy groups and trans-olefin structure of the parent stilbene skeleton contribute to the inhibitory potency of hydroxystilbene for tyrosinase activity.

- Palumbo A.
Melanogenesis in the ink gland of Sepia officinalis. Pigment Cell Res. 16(5):517-22, 2003.
Among the various melanin-producing systems, the ink gland of the cuttlefish (Sepia officinalis) has traditionally been regarded as a most convenient model system for the studies of melanogenesis. The ink gland is a highly specialized organ with immature cells in the inner portion, from where the cells gradually mature, migrate towards the outer portion of the gland and become competent to produce melanin giving rise to particulate melanosomes. When cell maturation is complete, melanin is secreted into the lumen of the gland, accumulated into the ink sac and ejected on demand. Biochemical studies carried out over the past two decades have shown that the ink gland contains a variety of melanogenic enzymes, including tyrosinase, a peculiar dopachrome rearranging enzyme (which catalyses the rearrangement of dopachrome to 5,6-dihydroxyindole) and a peroxidase (presumably involved in the later stages of melanin biosynthesis). These enzymes are functionally interactive in close subcellular compartments of ink gland cells and appear to act in a concerted fashion during the process of melanogenesis in the mature portion of the gland. More recent studies have revealed that ink production and ejection are affected and modulated by the N-methyl-D-aspartate (NMDA)-nitric oxide (NO)-cyclic GMP (cGMP) signalling pathway. Glutamate NMDA receptor and NO synthase, the enzyme responsible for the synthesis of NO, have been detected by biochemical and immunohistochemical techniques in immature ink gland cells. Stimulation of NMDA receptors caused a marked elevation of cGMP levels, activation of tyrosinase and increased melanin synthesis in the mature portion of the gland, via the NO-guanylyl cyclase interaction. This signalling is also present in different regions of the nervous system in Sepia and in certain neural pathways controlling contraction of the ink sac sphincters and wall muscle in the ejection mechanism. Overall, these and other findings allowed elaboration of an improved model of melanin formation in Sepia, which underscores the complex interplay of melanogenic enzymes and regulatory factors, highlighting both the similarities and the differences with melanogenesis in mammals.

- Panzella L, Napolitano A, d'Ischia M.
Oxidative conjugation of chlorogenic acid with glutathione. Structural characterization of addition products and a new nitrite-Promoted pathway. Bioorg Med Chem. 11(22):4797-805, 2003.
Chlorogenic acid (1), a cancer chemopreventive agent widely found in fruits, tea and coffee, undergoes efficient conjugation with glutathione (GSH), in the presence of horseradish peroxidase/H(2)O(2) or tyrosinase at pH 7.4, to yield three main adducts that have been isolated and identified as 2-S-glutathionylchlorogenic acid (3), 2,5-di-S-glutathionylchlorogenic acid (4) and 2,5,6-tri-S-glutathionylchlorogenic acid (5) by extensive NMR analysis. The same pattern of products could be obtained by reaction of 1 with GSH in the presence of nitrite ions in acetate buffer at pH 4. Mechanistic experiments suggested that oxidative conjugation reactions proceed by sequential nucleophilic attack of GSH on ortho-quinone intermediates. Overall, these results provide the first complete spectral characterization of the

adducts generated by biomimetic oxidation of 1 in the presence of GSH, and disclose a new possible nitrite-mediated conjugation pathway of 1 with GSH at acidic pH of physiological relevance

- Penalver MJ, Rodriguez-Lopez JN, Garcia-Ruiz PA, Garcia-Canovas F, Tudela J.
  Solvent deuterium isotope effect on the oxidation of o-diphenols by tyrosinase. Biochim Biophys Acta. 1650(1-2):128-35, 2003.
  A solvent deuterium isotope effect on the catalytic affinity (K(m)) and rate constant (k(cat)) of tyrosinase in its action on 4-tert-butylcatechol (TBC) was observed. Both parameters decreased as the molar fraction of deuterated water in the medium increased, while the k(cat)/K(m) ratio remained constant. In a proton inventory study, the representation of k(cat)(f(n))/k(cat)(f(0)) and K(m)(f(n))/K(m)(f(0)) vs. n (atom fractions of deuterium) was linear, indicating that, of the four protons transferred from the two molecules of substrate and which are oxidized in one turnover, only one is responsible for the isotope effects. The fractionation factor of 0.64+/-0.02 contributed to identifying the possible proton acceptor. Possible mechanistic implications are discussed.

- Plonka PM, Slominski AT, Pajak S, Urbanska K.
  Transplantable melanomas in gerbils (meriones unguiculatus). II: melanogenesis. Exp Dermatol. 12(4):356-64, 2003.
  We characterized the melanogenic apparatus in a family of transplantable gerbil melanomas (melanotic and amelanotic) using a combination of biophysical, ultrastructural and biochemical methods. Melanotic melanomas produced pure eumelanin but in vesiculo-globular melanosomes ('pheomelanosomes'); the eumelanosomes, characteristically ellipsoidal in shape with fibrillar or fibrillo-lamelar matrix, were never noticed. Melanotic melanomas also had significant tyrosinase activity and Zn, Pb/S, Ca and P content; all higher than in the amelanotic variants. The amelanotic variant, which was devoid of melanin pigment and melanosomes, had clearly detectable tyrosinase activity (albeit at 20% of that in the melanotic variant). Thus, with these multidirectional approaches we demonstrate that pure eumelanin can be synthesized in organelles ultrastructurally defined as pheomelanosomes, but a defect in the formation of melanosomes can prevent in vivo melanin synthesis despite the presence of detectable tyrosinase activity. We conclude that this melanoma system provides an excellent experimental model for the study of molecular components determining pheo- and/or eumelanogenesis. The information generated can be used for defining the roles of melanogenesis and of tyrosinase expression in the regulation of melanoma behavior and the effect of their modification on the course of the disease.

- Riley PA.
  Melanogenesis and melanoma. Pigment Cell Res. 16(5):548-52, 2003.
  Melanins are the principal surface pigments in vertebrates and, in humans, play a major role in photoprotection. Although the product (melanin) has a mainly protective function in the skin, the process of melanogenesis represents a potential cellular hazard and is confined to special membrane-limited organelles (melanosomes) in a set of specialized dendritic cells (melanocytes) which synthesize the pigment and transfer it to recipient cells. Malignant melanocytes tend to exhibit up-regulated melanogenesis and defective melanosomes. These features suggest ways in which anti-melanoma therapy may be specifically targeted. Two general chemotherapeutic modalities are considered: 1 The 'Achilles heel' approach in which the generation of reactive quinones capable of leaking into the cytosolic compartment and causing structural and functional derangement is encouraged by the use of analogue substrates. 2 The 'Trojan horse' approach, in which a cytotoxic agent is selectively released by a tyrosinase-dependent mechanism.

- Russo GL, De Nisco E, Fiore G, Di Donato P, d'Ischia M, Palumbo A.
  Toxicity of melanin-free ink of Sepia officinalis to transformed cell lines: identification of the active factor as tyrosinase. Biochem Biophys Res Commun. 308(2):293-9, 2003.
  The melanin-free ink of the cephalopod Sepia officinalis is shown to contain a heat labile proteinaceous component toxic to a variety of cell lines, including PC12 cells. Gel filtration chromatography indicated that the toxic component was concentrated in those fractions eluted at a molecular weight higher than 100 kDa and exhibiting the highest tyrosinase activity. SDS-PAGE analysis of the active fractions displayed a single major band migrating at an approximate molecular weight of 100 kDa, identical with that of the single tyrosinase band in the melanin-free ink. These data unambiguously demonstrated the identity of the toxic component with tyrosinase. Treatment of purified Sepia as well as of mushroom tyrosinase with an immobilized version of proteinase K resulted in a parallel loss of tyrosinase activity and cytotoxicity. Sepia apotyrosinase was ineffective in inducing cytotoxicity in PC12 cells. Purified Sepia tyrosinase was found to induce a significant increase in caspase 3 activity in PC12 cells, leading eventually to an irreversible apoptotic process. Overall, these results disclose a hitherto unrecognized property of tyrosinase that may lead to a reappraisal of its biological significance beyond that of a mere pigment producing enzyme.

- Valero E, Varon R, Garcia-Carmona F.
  Catalytic oxidation of acetaminophen by tyrosinase in the presence of L-proline: a kinetic study. Arch Biochem Biophys. 416(2):218-26, 2003.
  A kinetic study of acetaminophen oxidation by tyrosinase in the presence of a physiological nucleophilic agent such as the amino acid L-proline is performed in the present paper. The o-quinone product of the catalytic activity, 4-

acetamido-o-benzoquinone, becomes unstable through the chemical addition of L-proline, in competition with the nucleophilic addition of hydroxide ion from water. In both cases, the catechol intermediate, 3(')-hydroxyacetaminophen, is generated, as can be demonstrated by liquid chromatography. When the effect of the presence of the nucleophilic agent on the time course of the enzymatic reaction was kinetically analyzed, it was seen to decrease the duration of the lag period and increase the steady-state rate. Rate constants for the reaction of 4-acetamido-o-benzoquinone with water and L-proline were also determined. The results obtained in this paper open a new possibility to acetaminophen toxicity, that has been attributed hitherto to its corresponding p-quinone, N-acetyl-p-benzoquinone imine.

- Xie LP, Chen QX, Huang H, Liu XD, Chen HT, Zhang RQ.
  Inhibitory effects of cupferron on the monophenolase and diphenolase activity of mushroom tyrosinase. Int J Biochem Cell Biol. 35(12):1658-66, 2003.
  Mushroom tyrosinase (EC 1.14.18.1) is a copper containing oxidase that catalyses both the hydroxylation of tyrosine into o-diphenols and the oxidation of o-diphenols into o-quinones. In the present study, the kinetic assay was performed in air-saturated solutions and the kinetic behavior of this enzyme in the oxidation of L-tyrosine and L-DOPA has been studied. The effects of cupferron on the monophenolase and diphenolase activity of mushroom tyrosinase have been studied. The results show that cupferron can inhibit both monophenolase and diphenolase activity of mushroom tyrosinase. The lag phase of tyrosine oxidation catalyzed by the enzyme was obviously lengthened and the steady-state activity of the enzyme decreased sharply. Cupferron can lead to reversible inhibition of the enzyme, possibly by chelating copper at the active site of the enzyme. The IC(50) value was estimated as 0.52 microM for monophenolase and 0.84 microM for diphenolase. A kinetic analysis shows that the cupferron is a competitive inhibitor for both monophenolase and diphenolase. The apparent inhibition constant for cupferron binding with free enzyme has been determined to be 0.20 microM for monophenolase and 0.48 microM for diphenolase.

- Yamazaki S, Itoh S.
  Kinetic evaluation of phenolase activity of tyrosinase using simplified catalytic reaction system. J Am Chem Soc. 125(43):13034-5, 2003.

- Zhou H, Cadigan KM, Thiele DJ.
  A copper regulated transporter required for copper acquisition, pigmentation and specific stages of development in Drosophila melanogaster. J Biol Chem. 2003.
  The trace element copper (Cu) is required for normal growth and development, serving as an essential catalytic co-factor for enzymes involved in energy generation, oxidative stress protection, neuropeptide maturation and other fundamental processes. In yeast and mammals Cu acquisition occurs through the action of the Ctr1 family of high affinity copper transporters. Here we describe studies using Drosophila melanogaster to investigate the role of Cu acquisition through Ctr1 in normal growth and development. Three distinct Drosophila Ctr1 genes (Ctr1A, Ctr1B and Ctr1C) have been identified which have unique expression patterns over the course of development. Interestingly, Ctr1B, which is expressed exclusively during the late embryonic and larval stages of development, is transcriptionally activated in response to nutritionally-induced Cu deprivation and down regulated in response to Cu adequacy. The generation of Ctr1B mutant flies results in decreased larval Cu accumulation, marked body pigmentation defects that parallel defects in tyrosinase activity, and specific developmental arrest under conditions of both nutritional Cu limitation and excess. These studies establish that Cu acquisition through the Drosophila Ctr1B transporter is crucial for normal growth and in early and specific stages of metazoan development.

8. Melanosomes

(Dr. J. Borovansky)

Recent articles have addressed melanosomes from various points of view. Two first-class reviews (*Marks et al, Liu&Simon*) belong to recommended reading. Melanosome biogenesis and/or ultrastructure were studied by several authors (*Bagnara et al, Costin et al., Dell'Angelica, Liu et al, Marks et al, Prelovšek&Bulog*). Transfer of melanosomes to keratinocytes was characterized by *Boissy and Scott et al*, melanosome distribution in the keratinocytes of various ethnic origin by *Thong et al*. Role of melanosomal proteins in immunological reactions was discussed by *Mandelcorn-Monson et al* and *by Marks et al.*
Liu et al redemonstrated that isolation procedure has an impact on melanosome structure and physical properties. Papers describing molecular motors and melanosome transport have remained in minority this time (*Boissy, Fehrenbacher et al, Reilein et al*).

- Bagnara JT
  Enigmas of pterorhodin, a red melanosomal pigment of tree frogs.
  Pigment Cell Res 16(5): 510-516, 2003

Comments: Melanosomes observed in dermal melanophores of adult leaf frog have a unique structure: a small electrodense core of eumelanin surrounded by a concentric fibrous mass of wine-red pigment pterorhodin, unlike dermal melanophore melanosomes of larval leaf frogs that contain small eumelanin melanosomes.

- Boissy RE
  Melanosome transfer to and translocation in the keratinocyte.
  Exp Dermatol 12, suppl.2:5-12, 2003
  Comments: Review describing transfer of melanosomes from melanocytes to keratinocytes and its modulation by ultraviolet radiation, and distribution and degradation of the transferred melanosomes by the recipient keratinocytes.

- Costin GE, Valencia JC, Vieira WD, Lamoreux ML, Hearing VJ
  Tyrosinase processing and intracellular trafficking is disrupted in mouse primary melanocytes carrying the *underwhite (uw)* mutation. A model for oculocutaneous albinism (OCA) type 4. J Cell Sci 116(15): 3203-3212, 2003
  Comments: OCA4 is associated with mutations of membrane-associated transporter protein (MATP)gene. Molecular basis of hypopigmentation in OCA4 is disruption of tyrosinase processing and trafficking to the melanosome. Tyrosinase is abnormally secreted from the cells in immature melanosomes. The melanosomes remaining in melanocytes exhibit a disorganized structure of their internal fibres and contain only small amount of melanin.

- Dell'Angelica EC
  Melanosome biogenesis: shedding light on the origin of an obscure organelle.
  Trends Cell Biol 13(10): 503-506, 2003
  Comments: Brief review, summarizing recent work of the groups of Marks and Raposo on the molecular changes of gp100/Pmel17 during melanosome ontogenesis, concludes that the release of Mα fragment from the lumenal domain of Pmel17, mediated by a proprotein convertase, is a key event in the formation of melanosomal striations. Clear colour illustrations are the salient feature of the article; its deficiency is lack of original (primary) citations (replaced by later secondary references), particularly in the paragraph devoted to stages of melanosome ontogenesis.

- Fehrenbacher KL, Boldogh IR, Pon LA
  Taking the A-train: actin-based force generation and organelle targeting.
  Trends Cell Biol 13(9): 472-477, 2003
  Comments: General review discussing both myosin-dependent and myosin-independent mechanisms for actin-based intracellular organelle/cargo movement in various cells and also the processes underlying targeting of various actin-dependent force generators to their cargo. Text is accompanied by clear schemes.

- Liu Y, Kempf VR, Nofsinger JB, Weinert EE, Rudnicki M, Wakamatsu K, Ito S, Simon JD
  Comparison of the structural and physical properties of human hair eumelanin following enzymatic or acid/base extraction. Pigment Cell Res 16(4):355-365, 2003
  Comments: Comparative study of hair melanosomes isolated by harsh chemical and mild enzymatic procedures, demonstrated again that the conditions of the isolation procedure had a significant effect on the structure (documented by scanning electron microscopy and atomic force microscopy pictures) and physical properties of the separated melanosomes and their pigment moiety . (cf. *Borovanský et al / ČLČ 111, 1972, 218)*

- Liu Y, Simon JD
  Isolation and biophysical studies of natural eumelanins: Applications of imaging technologies and ultrafast spectroscopy. Pigment Cell Res 16(6): 606-618, 2003
  Comments:Excellent review in which an „evergreen" issue- the influence of isolation procedures on the resulting structure and properties of melanosomes/melanin was examined. By means of modern image techniques qualitatively new data on the ultrastructure of melanosomes and eumelanin assemblies were obtained. Generation of ROS was shown to be dependent upon the degree of melanin aggregation. Ultrafast laser spectroscopy studies of melanosomes extended our knowledge on mechanism by which melanin dissipates absorbed light energy.
  Reading the two papers of Liu&Simon reminded me of the importance to adhere to terminology strictly distinguishing between melanin-melanoprotein-melanosome, as recommended by *Duchon,Fitzpatrick &Seiji (1967-68 Year Book of Dermatology).*

- Mandelcorn-Monson RL, Shear NH, Yau E, Sambhara S, Barber BH, Spaner D, DeBenedette MA
  Cytotoxic T lymphocyte reactivity to gp100, MelanA/MART-1 and tyrosinase, in HLA-A2-positive vitiligo patients. J Invest Dermatol 121(3): 550-556, 2003
  Comments: Melanosomal proteins are known to be presented also on the cell surface (*see e.g. Marks et al/Immunol Res 271,2003,17*) with a potential to be involved in immunological reactions. This paper has brought a further support to the concept of immunopathologic mechanisms in vitiligo – in 15 of 17 patients with active disease antigen-specific T lymphocyte reactivity to gp100 peptides (but not to MelanA/MART-1 neither to tyrosinase peptides) was observed.

- Marks MS, Theos AC. Raposo G
  Melanosomes and MHC class II antigen-processing compartments. A tinted view of intracellular trafficking and immunity. Immunologic Res 27(2-3), 1-17, 2003
  Comments: Excellent review deserving to be ranked among the „top 5“ papers of 2003. It focuses on recent insights into melanosome biogenesis and their impact on understanding the formation of antigen-processing compartments and the presentation of melanosomal proteins as tumour-associated antigens.

- Prelovšek PM, Bulog B
  Biogenesis of melanosomes in Kupffer cells of *Proteus anguinus* (Urodela, Amphibia).
  Pigment Cell Res 16(4): 345-350
  Comments: The ultrastructural characteristics and size of melanosomes observed during their biogenesis in liver pigment cells of the cave salamander are described. Almost spherical melanosomes and premelanosomes were grouped in clusters delineated by a single membrane.

- Prem P, Dube KJ, Madison SA, Bartolone J.
  New insights into the physicochemical effects of ammonia/peroxide bleaching of hair and Sepia melanins. J Cosmet Sci 54(4):395-409, 2003.
  Comments: Isolated human hair melanosomes were compared with Sepia ink granules as for their size, ultrastructure, bleaching upon NH_3/H_2O_2 treatment and breakdown of melanosomes after prolonged treatment with ammonia. Sepia melanosomes were found to be more resistant to chemical attacks. The authors have suggested that ammonia helps to release melanin nanoparticles out of melanosomes making them more susceptible to oxidative attack by hydrogen peroxide. (*cf. Elleder&Borovanský/ Histochem J 33,2001,273, Borovanský&Elleder Pigment Cell Res 16,2003,280*)

- Reilein AR, Serpinskaya AS, Karcher RL, Dujardin DL, Vallee RB, Gelfand VI
  Differential regulation of dynein-driven melanosome movement.
  Biochem Biophys Res Commun 309(3): 652-658, 2003.
  Comments: Cytoplasmic dyneins, multisubunit microtubule motors, are thought to provide a means for independent movement of different organelles. Dynein-driven transport of melanosomes in Xenopus melanophores was not accompanied by the change of localization of mitochondria which suggests that melanosomes bear a dedicated form of dynein. Melanosome aggregation was specifically blocked after injection of an antibody specifically recognizing one of the three immunologically different dynein light chains , namely that one which fractionates with melanosomes.

- Scott G, Leopardi S, Parker L, Babiarz L, Seiberg M, Han R
  The proteinase-activated receptor-2 mediates phagocytosis in a Rho-dependent manner in human keratinocytes. J Invest Dermatol 121(3): : 529-541, 2003
  Comments: Signalling mechanisms involved in the PAR-2-dependent phagocytosis of melanosomes in human keratinocytes were studied. The phagocytosis was Rho-dependent because inhibition of Rho or its effector Rho kinase inhibited the PAR2-mediated phagocytosis. PAR2 activation either through PAR2-activating peptides or via trypsinization elevated cAMP in keratinocytes.

- Thong HY, Jee SH, Sun CC, Boissy RE
  The patterns of melanosome distribution in keratinocytes of human skin as one determining factor of skin colour. Brit J Dermatol 149(3): 498-505, 2003
  Comments: Melanosome distribution in keratinocytes of various ethnic origin was studied by means of electron microscopy. As expected from previous studies melanosomes in Caucasian keratinocytes were mostly clustered ,whereas those in dark skin mostly individual. In Asian skin keratinocytes melanosomes were distributed partly individually, partly in groups. Melanosome size descended according to the ethnic origin in the following order: dark skin – Asian skin- Caucasian skin.

9. Melanoma experimental, Cell culture

()

ANNOUNCEMENTS & RELATED ACTIVITIES

Calendar of events

2004 1st International Meeting on Neurobiology of the skin

Münster, Germany, February 13-15

Contact: Mrs Sabine Grünig
Dept of Dermatology
University of Münster
Von-Esmarch-Strasse 58
D - 48149 Münster
Phone: + 49-251-835 6517 Fax : +49-251-835 8579
E-Mail: neurobioskin@uni-muenster.de

2004 14th International Congress on Photobiology

Jungmoon, Jeju (Cheju), Korea June 10-15

2004 XIIth Annual Meeting of the PanAmerican Society for Pigment Cell Research

Orange County, California, USA, June 24-27

Organizers:Dr. Frank MEYSKENS (UC-Irvine) and Dr. Rogers BOWERS (Cal State-LA)
Contact: Dr Frank MEYSKENS
E-mail: flmeyske@uci.edu

2004 34th European Society for Dermatological Research

Vienna, Austria, September 9-11

Contact: AIMS International Congress Services
Mariannengasse 32
Au - 1090 Vienna
Tel: +43 1 402 77 55 – 97/-38 Fax: +43 1 402 77 31
E-mail: esdr2004@ahr-aims.com
Web: www.esdr.org

2004 International Skin Cancer Conference

Zurich, CH, July 22-24

President: Günter Burg; **Secretary:** Reinhard Dummer & Frank O. Nestle
Contact: Reinhard Dummer
Dept. of Dermatology, University Hospital of Zurich
Gloriastrasse 31
CH - 8091 Zürich
Phone: +41 1 255 88 37 Fax: +41 1 255 44 03
E-mail: nicole.brunner@usz.ch
Web : http://www.skincancer.ch

2004 XIIth Meeting of the ESPCR

Paris, France, September 22-25

Contact: Dr. Lionel LARUE
E-mail: Lionel.Larue@curie.fr
Congress Secretariat :
Teranga
89 rue Damrémont
F - 75018 Paris
tel : +33-1-44-92-36-36 fax :+ 33-1-44-92-36-30
E-mail : info-espcr2004@curie.fr
Web site: http://espcr2004.curie.fr/

2004 Perspectives in Melanoma VI

Miami, Florida, November 13-14

Contact: IMEDEX
70 Technology Drive
Alpharetta, GA 30005-3969 USA
Tel +1 (770) 751 7332 Fax: +1 (770) 751 7334
E-mail: meetings@imedex.com
Web: www.imedex.com

2004 18th Annual Meeting of the Japanese Society for Pigment Cell Research

Kumamoto City, Japan, November 27-28

Chair: Prof. Tomomichi Ono of Kumamoto University
Contact: Dr Toshiro Kageshita

2005 8th International Conference on Solar Energy and Applied Photochemistry

Luxor/Aswan, Upper Egypt, Photoenergy Center, February 20-26

Contact: Dr. Sabry Abdel-Mottaleb
Professor of Chemistry, Director, Photoenergy Centre
Fac. of Science, Ain Shams University,
Abbassia, 11566 Cairo, Egypt
Cellular: + 2012 216 9584 Fax: + 202 484 5941 OR + 202 634 7683
E-mail: solar05@photoenergy.org
Homepage: http://www.photoenergy.org

2005 XIVth International Pigment Cell Conference (IPCC)

Reston, Virginia, USA, September 18-23

Contact: Dr. V. HEARING
E-mail: hearingv@nih.gov
Web page: http://www.ipcc.info/

2006 XIIIth Meeting of the ESPCR

Barcelona, Spain

ESPCR GENERAL ASSEMBLY

Wednesday, September 17, 2003

Ghent, Belgium

Time: 18:00.
Place: Aula of the University, Ghent

1- Opening of the General Assembly

The Assembly was opened by D. Bennett who welcomed all attendants.

2- Approval of the minutes of ESPCR General Assembly in Egmond aan Zee

The Minutes of the previous General Assembly, held in Egmond aan Zee, were approved and signed by the President.

3- Secretary's report

This was delivered by J.C. García-Borrón. During this year, the relevant activities that concerned the Secretary were the preparation of a Meeting Report, the election of a new ESPCR Treasurer, and the preparation of a series of activities "in memoriam" of Prof. G. Prota.

Following a decision of the Council taken in 2001, a Meeting Report is being prepared with the close collaboration of the Meeting Organizers, of chair persons for each one of the sessions in this meeting, and of the ESPCR Bulletin Editor. Chair persons or relevant experts have been contacted and agreed to prepare a summary of the presentations in their sessions. These contributions will be collected by the Secretary, and the resulting report will be published in the December issue of the ESPCR Bulletin.
The Secretary thanked the Organizers for their availability and for the strong and enthusiastic input of the Ghent group, as well as all the contributors.

P. Verrando, elected Treasurer for the 2003-06 period, communicated in January that he was not going to be able to take on the Office. A new call for candidates was launched on February 20. The only candidate duly nominated, was J. W. Lambert, from Ghent University. J. Lambert will take on the Office at the end of the XIth ESPCR Meeting. This was already communicated to the membership in an announcement published in the August issue of the ESPCR Bulletin. Following the different elections for Offices and Council held during 2002 and 2003, the compositions of the Offices and Council, effective at the end of this Meeting are:

Offices

President, Jose Carlos García-Borrón (Murcia); Secretary, Jean Marie Naeyaert (Ghent);
Treasurer: Jo Lambert (Ghent).

Council

F. Beermann (Lausanne), D. Bennett (London), J.C. Garcia-Borron (Murcia), G. Ghanem (Brussels), C. Goding (Oxted), J. Lambert (Ghent), L. Larue (Paris), J. M. Naeyaert (Ghent), M. Picardo (Rome), N. Smit (Leiden) A. Taïeb (Bordeaux).

Early this year, the pigment cell community was shocked by the sad news of the death of Prof. G. Prota, one of the founders of the ESPCR, and its Inaugural President. A series of activities have been prepared to honour his memory. An obituary was written by P. Riley, and published in the April issue of the ESPCR Bulletin (vol. 45, pp 1231-1233). Another obituary by M. d'Ischia appeared in Pigment Cell Research (vol. 16, 3, pp 170-171). A scientific session in the XIth ESPCR Meeting was dedicated to his memory (G. Prota Session on Chemistry of Melanins).
On behalf of the ESPCR, the Secretary expressed his gratitude to those who contributed to make possible the memorial activities.

D. Bennett thanked the Secretary for his report and moved on to the next item.

4- Treasurer's report

L. Larue, presented data on membership. The number of members in September 2003 is 185. During this year, the Society welcomed 10 new regular members, and 5 new student members. As of September, 121 members paid their membership fees. This is a higher percentage of the membership than in previous years.
L. Larue then described the incomes and outgoings, as follows (data given in euros):

Balance carried over from 2001:	4,031.54
Income 10/02-09/03	
Member subscriptions 2002	1,878.00
Member subscriptions 2003	7,146
Pigment Cell Research subscriptions	4,350.00
Donations	0,070.00
Total income	13,444.00
Outgoings 9/02-9/03	
International Federation of Pigment Cell Societies, subscriptions for 2003	2,626.54
Ghent Meeting and Fritz Anders Lecture	4,000.54
Bulletin and web costs	0,210.00
Bank charges CCF	0,409.83
Bank charges Natwest	0,050.00
ESPCR Travel Awards	1,990.00
Pigment Cell Research Subscriptions	4,070.00
Credit card machine (running budget)	0,165.05
Total outgoings	13,521.96
Balance at 09/02	3,953.58

This positive balance is stable as compared to last year. The Treasurer's report was unanimously accepted without further questions. D. Bennett thanked the Treasurer for his work.

5- ESPCR Bulletin and Web site report

G. Ghanem reported that there were no major news concerning the web site, other than some updates performed with suggestions from the membership, for which he was thankful. A new password was selected for the next year (Paris). The running costs of the ESPCR Bulletin have been cut following the decision to discontinue the printed version. Some changes in contents are foreseen for next year, concerning the sections in the Review of the literature.
D. Bennett thanked Ghanem for his important contribution to the Society.

6- Election of ESPCR Officers and new Council composition

This topic was already treated in the Secretary report, and D. Bennett moved to the next item.

7- New composition of the ESPCR Awards and Travel Awards Committees

The activities of the Travel Awards Committee were summarised. Ten applications were received, of which four received total or partial funding. The President also informed that, following the resignation of two members, C. Goding and J.C. García-Borrón, a new composition of the Committee was decided by the Council: M. Picardo, L. Larue and F. Beermann as Chairman. The President thanked the new members, and the resigning members for their work. D. Bennett communicated that a new ESPCR Awards Committee must be formed, following the death of G. Prota, and the resignation

of J.C. García-Borrón. This will be done in due time, since there are no urgent duties for the Committee.

8- Any other business

D. Bennett informed that the next Pigment Cell Research Editor, starting January 2005, will be C. Goding. The President also reminded that the next ESPCR Meeting will be held in Paris, in September 22-25, 2004. Then, D. Bennett invited Lluis Montoliu to communicate that he is willing to organize the 2006 Meeting in Spain, very likely in Barcelona.

9- Close of Assembly

With no further questions from the audience or other matters to discuss, the General Assembly was closed by D. Bennett.

EUROPEAN TASK FORCE ON VITILIGO PROJECT
GHENT, 19 SEPT 2003
Minutes of the Meeting (by Dr M. Picardo)

The principle of a European Task Force on Vitiligo was discussed at previous meetings between Alain Taïeb and Mauro Picardo, based on the model of the task force created in Bordeaux in 1990 for atopic dermatitis (ETFAD), which hold now annual meetings at the EADV, but which started with a small group of motivated investigators who met initially on an informal basis at European and International meetings to develop common projects, especially the SCORAD project (Currently 110 international references on Pubmed when the SCORAD keyword is hit). Dorothy Bennett, president of the IFPCS and organizer of a very successful vitiligo conference last May in London, was informed of this project and supported the initiative, which could foster better interactions between clinical and basic research in the pigment cell community. The project was also supported by J M Naeyaert who accepted to host a roundtable discussion during the 11th ESPCR. Clinical investigators active in the field representing various European countries were invited including A Alomar (Barcelona), M Böhm (Münster), Y Gauthier and A Taïeb (Bordeaux), D Gawkrodger (Sheffield), R Kaufmann (Frankfurt), S Moretti (Florence), JM Naeyaert and N van Geel (Ghent), M Olsson (Uppsala), G Orecchia (Pavia), JP Ortonne (Nice), M Picardo (Rome), K Schallreuter (Bradford), W Westerhof and D Njoo (Amsterdam).

Most invited panellists could come (photograph) and adhered enthusiastically to the creation of the task force. A lunch meeting was arranged by Jean Marie Naeyaert and his coworkers with the generous sponsoring of Novartis and Pierre Fabre companies. The first part of the meeting took place in a previous abbey dormitory now part of the beautiful university buildings in the historical centre of the city. After a short presentation of their activities and interests in the field, decided to meet for a one day meeting in January to discuss the major points put on the agenda namely consensus definition of disease; design biometric tools to assess disease severity/stability; and if possible, derive a consensus scoring system

A working method was proposed by A Taïeb based on a preliminary discussion with Y Gauthier and the experience gained in the field of atopic dermatitis. The principle to discuss with the help of a biomedical statistician a descriptive set of data derived from a dozen of centers (including each 10-15 patients) would be the basis of the next ETFV meeting, with the objective to write a position paper in due time.

The joint organization of a workshop with American and Asian colleagues at the next IPCC in Washington (Sept 2005) including a progress report of the ETFV initiative was discussed with D Bennett who could attend the meeting. This issue will be put on the agenda of future meetings of this new ETFV.