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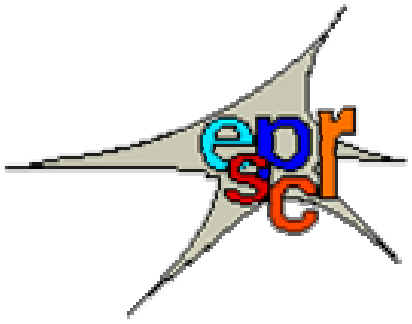

EUROPEAN SOCIETY FOR PIGMENT CELL RESEARCH *BULLETIN*

N° 56 - Dec 2006

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: 32-2-541.33.49 E-Mail:gghanem@ulb.ac.be

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Letter to ESPCR Members

Dear friends,

It was very nice seeing you all at the Barcelona meeting, a special one for me in taking over as President of the ESPCR. I am endebted to you all for electing me, despite being a somewhat daunting task! It's also a great honour to be the 2nd Italian President, following in the footsteps of the late Prof Giuseppe Prota, the first ESPCR President, whom I'm sure all of you remember well I am also fortunate to be ably assisted by 2 very dedicated members of the ESPCR society, Dr Lionel Larue as Secretary and Prof Jo Lambert as Treasurer, whose term of office has been extended due to the excellent job she did in the last triennium.
I would like to thank the outgoing President Prof Jose-Carlos Garcia Borron who has worked tirelessly over the past 3 years in bringing the ESPCR to where it is today. Thanks also to the outgoing members of the council and I'd like to welcome all the newly elected council members on board. A special mention to Prof Jean Marie Naeyaert whom we hope to see back on the ESPCR council very soon.

I am taking over the presidency of the society at a time when the ESPCR is in a very healthy state, borne out by the very sucessful meeting in Barcelona, whose success was due to the excellent organisation by Dr Lluis Montoliu and the quality of the papers presented. This meeting is a reflection of how the ESPCR is growing and the quality of scientifc research that it attracts. We must all be dedicated to maintaining this high standard.

The ESPCR now has an "official " registered website and special thanks goes to Dr Lluis Montoliu and Prof Ghanem Ghanem for setting this up. I'd also like to thank Prof Ghanem for his work on improving and maintaining the website, as well as producing this bulletin.

Pigment Cell Research, the official journal of the ESPCR is improving all the time which is due to the excellent work of the first European Editor – in Chief, Dr Colin Goding who has introduced a number of changes since he took over and the journal has become ever more appealing with a new design and interesting features. The ESPCR must remain committed to supporting and promoting the journal in order to reap the benefits mutually.

For the future we all want to see the society becoming bigger and better and we can do this in a number of ways. All of us have to make a concerted effort to spread the word about ESPCR and the many advantages it can offer, which we are all au fait with, including the many great friends we have all made. The open friendly spirit and the ease with which we collaborate is characteristic of the society and something that makes it special.

Organising ESPCR workshops and symposiums at European and International Scientific Meetings will enable members to meet more often and interact with other Scientific Societies, rather than just one major meeting every year Presently we are organising a small workshop at the next ESDR meeting in Zurich.

This will benefit us in a number of ways: get more publicity for the ESPCR and hopefully increase coperation between researchers even more. In addition to this we will now contact the authors of papers who are not ESPCR members, whose work is reviewd in the bulletin, informing them about the review and using it as an opportunity to invite them to join us.

For ease of information between Institutions and researchers, setting up a forum on the ESPCR website would be another possible way to encourage more collaboration and facilitate research exchanges.

Preparations are underway for next year's meeting which is being organised by Prof Rosa Cicero and we can all expect a warm welcome in Bari and enjoy some southern Italian hospitality. We are all looking forward to an eventful 2007 meeting with an excellent program lineup.

I'd like to take this opportunity to thank you once again for choosing me as President and to wish you a very happy Christmas and a peaceful 2007.

Mauro Picardo
ESPCR President

Meeting Report (Barcelona, sept 2006)

(Prepared by session Chairs)

Session I: Developmental biology of pigment cells

Chairs: Lionel Larue, Lidia Kos

Session I had 3 invited speakers and 3 selected presentations, which were chosen from the submitted abstracts. The studies presented used the mouse as a model to unravel novel developmental processes in pigment cell biology. The session was opened by a lecture of **Takahiro Kunisada (Gifu, Japan)** who reported his last results on the differentiation of ES cells towards melanocytes. Strong arguments were presented to show that ES cells were first differentiating into neural crest cells before differentiating into neurons, glial cells or melanocytes. Different mutant ES cells were successfully established and differentiated. Melanocyte precursors could be identified as c-Kit and/or Sox10 positive cells. The second presentation by **William J Pavan (Bethesda, USA)** focused on the Sox10 transcription factor. A large genetic ENU chemical mutagenesis screen affecting melanocytes and peripheral nervous system development on a Sox10 background was performed. This strategy has allowed the authors to discover genes acting in a synergistic manner with Sox10 or affecting its expression. Very exciting phenotypes and genes were presented. The functions of two other transcription factors in pigmentation were described in the third presentation. The general role of the TATA-binding protein TBP and 14 TBP-associated factors TAFs was presented by **Irwin Davidson (Illkirch, France**). One of the TAF proteins, TAF4, is important in the retinoic and TGF-b signalling to promote cell proliferation. The roles of TAF4 and TBP were tested in vivo by specifically deleting these genes in melanocytes. In both cases, the coat colour of mutant mice was affected. The absence of TAF4 in melanocytes led to hypopigmentation and white belly while the absence of TBP results in a complete lack of coat pigmentation. **Lidia Kos (Miami, USA)** described the phenotype of transgenic mice that express endothelin 3 in an inducible manner under the control of the keratinocyte 5 promoter. The expression of endothelin 3 from keratinocytes led to a dramatic hyperpigmentation phenotype due to an increase in the number of precursors and differentiated melanocytes in the dermis. The phenotype shares some characteristics with human conditions of dermal melanocytosis such as blue nevi. The generation of the hyperpigmentation phenotype was dependent on the early expression of the transgene and was capable of partially rescuing the pigmentation phenotypes of Kit and lethal yellow mutants. **Luisa Lanfrancone (Milan, Italy)** introduced a protein, RalP, which has been recently related to cells of melanocytic origin, in particular, vertically growing and metastatic melanomas. RaLP is a member of the Shc family of adaptor proteins. Silencing of RaLP protein expression in metastatic melanomas inhibited cell migration without affecting cell proliferation. Analysis of RaLP expression by in situ hybridization during mouse embryonic development revealed a specific positivity of the territories that are site of neurogenesis in vivo. In vitro experiments showed high levels of RaLP protein product in neural stem cells and migrating melanoblasts, which decrease in the differentiated, resident melanocytes, suggesting a possible role for RaLP in the migration of developing neural crest cells. Mouse embryonic stem cells do not express RaLP transcripts when grown in culture, but its expression is present during the organization of the cells into embryoid bodies, declining progressively during the differentiation towards the neuronal lineage. Finally, **Jean-Jacques Panthier (Paris, France**) presented the role of Notch in melanocytes as unveiled by loss-of-function and gain-of-function analyses. Melanoblasts emigrating from the neural crest were sensitive to both loss of Notch signalling and to misexpression of activated Notch. Loss of Notch signalling affected melanocyte stem cell renewal. By contrast, misexpression of activated Notch did not have an effect on these cells. The effects of activated Notch in melanocytes were shown to be mediated through RBP-J, i.e. the canonical Notch pathway.

Session II: Genetics of pigmentation

Chairs: Shigeki Shibahara, Colin Goding

The zebrafish has a number of advantages for the study of pigment cell biology, not least the presence of distinct pigment cell types. But what underlies the choice between once cell fate and another is poorly understood. **Robert Kelsh (Bath)** provided genetic evidence for a role of a zebrafish ortholog of Anaplastic Lymphoma Kinase (ALK), a proto oncogenic

receptor tyrosine kinase, in regulating the origin of iridophores. In the *shady* mutant, ALK expression is drastically reduced and the fish lack iridophores though there is no apparent effect on other neural crest-derived lineages including melanophores. The data presented provide substantial evidence that Shady/Alk may be implicated in both iridophore specification and proliferation. However, precisely how Shady/Alk mediates its effects are unclear at present. All structures that we can call eyes are in fact composed of at least two types of cells, light-sensitive photoreceptor cells, and light-absorbing pigment cells. The vertebrate eye contains two types of pigment cells, neural crest-derived choroidal, ciliary and anterior iris pigment cells, and neuroepithelium-derived retinal and posterior iris pigment cells. All these pigment cells share a critical dependence on the microphthalmia-associated transcription factor MITF, although they respond differently to MITF mutations: neural crest-derived pigment cells usually die, and neuroepithelium-derived pigment cells usually survive and in fact can change their developmental fate and develop, for instance, into a retina. While the cause of this differential response is not clear, it is conceivable that it is linked to the selective expression of distinct MITF isoforms. Indeed, MITF is a fairly complex gene, with at least nine different promoters giving rise to at least six different protein isoforms with distinct aminotermini. To address the question of which isoforms are expressed in the optic neuroepithelium during development, Bharti and collaborators (**Arnheiter lab, NIH**) used physical separation of the developing mouse eye (from embryos lacking potentially contaminating neural crest-derived melanocytes) into RPE and retina and determined by quantitative RT-PCR assays that A-MITF and several other mRNA isoforms are common to both retina and RPE, though their expression levels per cell are low. In contrast, the D-isoform is fairly exclusive to the RPE, and its expression level per cell is high. Thus, the seemingly complex network of many distinct isoforms can be substantially reduced in the RPE to a single isoform, D. Interestingly, CHX10, a retinal transcription factor responsible for down-regulation of MITF in the future retina, targets mostly the D promoter. Given these results, Bharti and collaborators knocked out the D promoter selectively and crossed the knock-out mice with CHX10 mutant mice which usually, by way of the missing downregulation of MITF in the retina, have a severely hypoplastic retina and very small eyes. Preliminary results indicate that the selective elimination of the D promoter partially normalizes eye size in the absence of CHX10. It remains to be shown, however, whether this partial rescue is simply due to a reduction in the levels of a major protein isoform, or whether this particular protein isoforms accesses specific target genes leading to specific cell biological responses during development. One of the major challenges to understanding Mitf function is to reveal the role of the plethora of modifications that modulate Mitf activity. These include phosphorylation by ERK and RSK as well as GSK3 and p38, ubiquitylation, sumoylation and acetylation. While cell based approaches will no doubt reveal how these modifications mediate or disrupt protein-protein interactions or capacity to bind DNA, it will be essential to complement these cell based studies with an understanding of how these modifications might affect the behaviour of Mitf and the melanocyte lineage during development. The traditional knock-in mutation strategy is difficult, time consuming and expensive. However **Eirikur Steingrimsson (Reykjavik)** reported an alternative approach, complementation of an Mitf-null mouse by making a transgenic using a BAC bearing the entire *Mitf* gene. Complementation with a WT *Mitf* BAC gave rise to black mice, indicating that complementation was working well. The advantage of the system was then exploited to knock in point mutations into the *Mitf* BAC at each of several sites of modification to give rise to mice with various Black/white pigmentation patterns. For example, mutation of the MAP kinase phosphorylation site Ser73 to alanine led to a failure to fully complement, while a double mutant S73 and S409 (the RSK phosphorylation site) gave a black mouse suggesting that modification on S73 and S409 may act antagonistically, a result that contrasts from conclusions drawn from cell based assays. No doubt the power of this system that enables rapid analysis of *Mitf* mutations will yield several insights into the role of these modifications in vivo, though as always careful analysis and interpretation of the phenotypes will be necessary. **Angel Garcia Diaz** and colleagues from Lluis Montoliu's laboratory in **Madrid** reported recent results on the dissection of the locus control region (LCR) of the *tyrosinase* gene. LCRs in general act to insulate downstream promoters from the potential inhibitory effects of heterochromatic spreading from neighbouring repressed genes or other heterochomatin regions. The *tyrosinase* LCR comprises at least two elements, a G-box and an AB box. Using a combination of in vivo assays, in vitro DNA-binding and chromatin immunoprecipitations, these authors were able to identify CTCF, a factor previously known to operate at the globin LCR as a key factor in mediating insulator function. CTCF was able to ChIP from the G box and from the 3' of the locus, perhaps indicating that it was participating in a 3-dimensional DNA looping event. The further dissection of the LCR should provide a major insight into the control of *tyrosinase* gene expression in a chromosomal context. **James Lister's** laboratory has focussed on using the zebrafish as a model system with its inherent advantage in terms of genetic tractability and ease of use. The presentatuon focused on the zebrafish mitfa encoded by the *nacre* gene, which would be the ortholog of the mammalian melanocyte specific Mitf-M isoform. Using a genetic screen three new hypomorphic *mitfa* alleles were isolated, two of which correspond to single point mutations in helix 1 of the bHLH-LZ DNA-binding and dimerisation domain, while the third affected a splice donor and thereby choice of splice sites necessary for correct mitfa function. The analysis of these mutants and further studies on chimeric Mitf proteins is ongoing. Reported Previous studies have suggested that pigmentation genes are differentially regulated in melanocytes and RPE and in transgenic mice, the promoter of the *Tyrp1* gene has been shown to drive detectable *lacZ* reporter gene expression only in RPE, even though this gene is also normally expressed in melanocytes. **Fabien Murisier** from Lausanne therefore searched for a putative melanocyte-specific regulatory sequence and demonstrated that a bacterial artificial chromosome (BAC) containing the *Tyrp1* gene and surrounding sequences was able to target transgenic expression to melanocytes and to rescue the $Tyrp1^{b}$ (brown) phenotype. They further demonstrated using both cell culture and transgenic mice experiments the presence of a Sox-10 inducible evolutionarily conserved sequence located at -15 kb which behaves as a melanocyte-specific enhancer. Although this element permitted expression in melanocytes, it was not sufficient to provide detectable lacZ expression to the RPE of transgenic embryos indicating that other regulatory elements are required. The presence of a distal *Tyrp1*

regulatory element, which specifies melanocyte-specific expression, supports the idea that separate regulatory sequences mediate gene expression in melanocytes and RPE.

Session III: Melanins and Melanogenesis
Chairs: Dr. Francisco Solano, Dr. Nico Smit

The session had 3 invited lectures and 3 selected presentations which were chosen from submitted abstracts. The session was opened by **Dr. Vince Hearing** (NIH, USA) who reported a collaborative study with other labs concerning the regulation of human skin pigmentation by UV irradiation. They used different ranges of phenotypes and protocols of MED doses, UV lamps and time to take biopsies. Using standardized chronic exposure protocols, in addition to visual evidences, they measured a set of melanogenic parameters including MITF expression, Pmel17, tyrosinase and melanin. Melanin increases only by 50% in spite of the dramatic change of visible skin pigmentation in Caucasians. Maximal levels for the different melanocyte differentiation markers were obtained from 1 week (MITF) to 5 weeks after treatment (Pmel17 and melanin). Localization of melanin in lower, middle and upper epidermis was also followed emphasizing the differentiation in the skin and the important role of pigment distribution and facultative tanning to provide photoprotection. DNA damage was visualized by antibodies against cyclobutane pyrimidine dimers (CPDs). Interestingly the increase in CPDs due to UV radiation in untanned skin and tanned skin (by solar simulated radiation) was almost the same indicating no additional protection by the acquired tan. **Dr. Jose C. García-Borrón** (Univ. Murcia, Spain) reported new data on the functional status and transport process of several MC1R variants. MC1R is very polymorphic, and some variants are associated with red and fair hair (the RHC phenotype). He presented studies on the most frequent RHC alleles, which are R151C, R160W and D294H, although some data on other mutations, such as T157A and R162P were also commented. The R151C, R160W and D294H variants showed reduced functionality but still considerable residual cAMP synthesis while the desensitization kinetics were reduced as compared to the WT. The retention of those variants in the ER is clearly different, e.g R151C is mostly retained but D294H mostly reaches the plasma membrane. The second intracellular loop of the MC1R structure is the major determinant of the maturation process in ER/Golgi. Phenotypic effects of RHC alleles do not correlate with their degree of functional loss. Heterodimers of two different alleles can be formed, and this could partially accounts for the lack of correlation. But surprisingly, Dr. Garcia-Borron showed that the highest degree of functional impairment corresponds to D294H which is efficiently processed through the secretory pathway, whereas other forms with relatively minor functional loss are recognized as aberrant (R151C or R160W). **Dr. Alessandra Napolitano** (Univ. Napoli, Italy) reported on new advances and perspectives on an old but still partially unsolved issue, the structural markers for pheomelanin analysis. It is well known that pheomelanins are the main pigment in red hair color phenotype, and those pigments show photosensitizing properties and low protection against sunlight. However, there are still some apparent discrepancies on these issues, and the actual structural determinants of that response are still unknown. Oxidative alkaline degradation of pheomelanin leads to TTCA (a tricarboxylic triazole) and BTCA (a carboxylated benzothiazole derivative). Dr. Napolitano presented that the improvement and simplification of the analytical procedure allows her group to identify new degradation products, possibly BTCA isomers that might be potential candidates to become new markers for prediction of high risk individuals against UV light. The relationship between the existence of those compounds and some MC1R variants alleles is currently underway. Furthermore, the current procedure offers the possibility to detect eumelanin and pheomelanin degradation products in one analytical run. The method has been proven useful for hair samples. As indicated in the discussion its use needs to be further proven for skin or cell culture samples. **Dr. A.L. Cook** (Inst. Molecular Bioscience, Brisbane, Australia) reported on the use in the analysis of melanogenesis of defined genotypes in SLC45A2/MATP and SLC24A5/NCKX5. The MATP protein has been associated with the degree of pigmentation in human skin, and the 374L allele seems to be highly associated with darker skin in comparison to the 374F variant. Similarly, the protein encoded by the human homologue to the zebrafish golden gene NCKX5 has a similar pattern at position 111, 111A allele associated to darker skin than 111T. He reported the establishment of a bank of primary human melanocytic cells from neonatal foreskin with characterization of those MATP and NCKX5 alleles in correlation to melanin content and expression of tyrosinase and other melanogenic genes. The contribution of the above mentioned gene variants on the process of melanogenesis is currently under study. Then, **Dr. Shosuke Ito** (Fujita Health Univ., Japan) presented some data related to the diffuse hyperpigmentation and increased oxidative stress in patients undergoing hemodialysis (HD). He examined serum levels of the pheomelanin precursor, 5-Cys-dopa and the degradation products of pheomelanin (3-AHP and 4-AHP) of which 3-AHP is also indicative of the presence of 3-nitrotyrosine. Some other oxidative stress markers were also measured in 16 patients with chronic renal failure during hemodialysis treatment and compared to healthy controls. Levels of these parameters were significantly elevated in patients, supporting that pheomelanin accumulates in the skin of those patients due to the high serum levels of 5-Cys-dopa because of the renal failure. Finally, Dr. **Victoria Maresca** (Ist. San Gallicano, Italy) presented some data about the strict association between melanin synthesis and catalase activity in 9 different primary cultures of human melanocytes. All biochemical parameters related to catalase, including mRNA, protein level and enzymatic activity, are directly associated to the degree of pigmentation and amount of melanin. On this basis, she postulated that pigmentation possesses two protective strategies against UV radiation, the formation of melanin as a radiation filter and the increase of catalase to eliminate hydrogen peroxide that possibly can also produce cellular damage by oxidative stress.

Session IV: Animal Models of Pigmentary Disorders and Biological Resources
Chairs: Dr F Beermann, Dr L Montoliu
Contributed by Dr F Beermann

The Session IV had 2 invited speakers (a third speaker had to resign shortly before the conference) and 3 selected presentations, which were chosen from the submitted abstracts. The session was opened by a lecture of **Maria Blasco (Madrid, Spain)** who reported on her work on telomere biology with emphasis on skin biology. Lack of telomeres (*Tert-/-*) in skin cells leads to hair loss, graying, an increased resistance to cancer and reduced wound healing. In contrast, keratinocyte-specific overexpression of Tert (*K5::Tert*) increases wound healing but renders mice more prone to skin cancer. Follow-up analyses showed that in hair follicles and skin, the number of stem cells decreases in function of the presence of telomerase (comparing *Tert-/-* to wildtype to *K5::Tert*), and that the longest telomeres are found in stem cells and thus provide a new marker for the niche. Using a quantitative chromosomal in-situ hybridzation (FISH), her group established telomere length topographic maps ("telo-mapping"), thus providing evidence that telomeres shortened with life in epidermal stem cells. Additional experiments using p16 and p53 knockout mice and overexpression of telomerase provided evidence that the presence of telomerase is a rate-limiting step for the age of a mouse. A different experiment was subject of the last 5 minutes of her talk. Here, Maria Blasco reported on mice overexpressing a telomere-binding protein (TRF2) in keratinocytes (*K5::TRF2*). This led to premature skin aging, hyperpigmentation (!) and an increased appearance of squamous cell carcinoma, a phenotype reminiscent of human Xeroderma pigmentosum. The phenotype is the consequence of defective DNA repair accompanied by a shortening of telomeres. **Elena Sviderskaya (London, UK)** started her presentation by an introduction to the functional genome cell bank where melanocyte, melanoma and keratinocyte cell lines are established and can be obtained (http://www.sgul.ac.uk/depts/anatomy/pages/ WTFGCB.htm). Currently, 265 different cell lines are available, including lines from mouse models of Hermansky-Pudlak-Syndrome or Griscelli-Syndrome, neural crest-like cells or human immortal melanocytes. Elena Sviderskaya then reported on her recent results on the relative roles of the p16/Rb and ARF/p53 pathways in the control of melanocyte senescence. Melanocyte cell lines derived from INK4a/ARF-/- mice, ARF-/- mice, p16INK4a-/- mice and p53-/- mice were compared with respect to proliferation. In contrast to what might be expected from ARF being a target of p53, ARF-/- cell lines do not behave as p53-/- cell lines and grow faster in the initial cultures. Thus, the activation of ARF is not entirely p53-dependent. **Anna Golovko (Uppsala, Sweden)** reported on attempts to identify the gene locus responsible for the *grey* coat color in horses. Apparently, the mutation appeared about 1000 years ago, and using "identical-by-descent" mapping, they could finally restrict the candidate region to ~700 kb. This interval contains 4 genes, which are all expressed in melanoma. Even though no coding mutation was identified, one non-coding mutation was linked to the phenotype. Further experiments to identfiy the gene defect using mouse experiments are currently under way. **Karine Schouwey (Epalinges, Switzerland)** reported on the role of the Notch signaling pathway in melanocytes. Using conditional knockout mice, she showed that both Notch1 and Notch2 receptors are involved in maintenance of the melanocyte lineage, and when deleted, lead to a dose-dependent and progressive hair greying caused by the loss of melanocyte stem cells. In contrast, non-follicular melanocytes and pigmentation in dermis (ear) and in the eye (choroid) seemed not to be affected by removal of Notch. **Susanne Kerje (Uppsala, Sweden)** reported on the efforts to isolate a gene for vitiligo using the Smyth line chicken. In this vitiligo model, melanocyte defects are present as well as autoimmune reactions to melanocytes. Genetic intercrosses with the vitiligo-resistant Brown chicken line were performed followed by SNP (single nucleotide polymorphisms) analysis to map the gene defect to chromosome 1.

Session V: Signalling pathways in melanocytes and melanoma cells
Contributed by C. Bertolotto and C. Jiménez-Cervantes.

The plenary session V had 3 invited lectures and 3 selected presentations. **Colin Goding** identified MITF as a regulator of the Diaphanous-related formin DIA1, a new MITF target. In melanoma cells, depletion of MITF, leads through DIA1 to reorganization of the actin cytoskeleton and through SKP2 to a p27-dependent G1 arrest that is accompanied with increased invasiveness via ROCK. **Robert Ballotti** also reported new MITF targets such as c-Met, the receptor of HGF that is involved in cancer cell survival and motility and Rab27,that is involved in melanosome movements in melanocyte. Stimulation of c-met by HGF in association with cAMP elevating agents protects normal melanocytes from apoptotic stimuli. In agreement with the results from Colin's lab, MITF silencing by siRNA changes melanocyte morphology and prevents melanosome transport to the dendrite tips. **Richard Marais** talked about MITF regulation by the MAPK/ERK. While MITF has been recently shown to be down-regulated by the oncogenic V600E B-Raf in normal melanocytes, in their transformed counterpart, ie melanoma cells, V600E B-Raf stimulates the transcriptional activity of the MITF promoter. Regulation of MITF promoter by V600E B-Raf involved the transcription factor Pax3. Therefore, it appears that V600E B-Raf controls both MITF stability through ERK activation and MITF phosphorylation and degradation and MITF transcription through PAX3. Next the 3 selected presentations. **Kimberley Beaumont** addressed the question of individual MCIR alleles function and cellular localization and its correlation with pigmentation phenotypes. She showed that the function of "R" and "r" MC1R alleles and its subcellular location in melanocytes correlates with its genetic association with pigmentation. Hence, MC1R variants could modify wild type MC1R localization and cAMP signaling through heterodimers formation, and this could explain the heterozygote effect on skin color and melanoma risk. **Keith Hoek** reported the existence of two melanoma groups. The first one is low metastatic, expresses MITF and is responsive to TGF- -mediated growth inhibition while the second is high metastatic, has low MITF and is not responsive to TGF- -mediated growth inhibition. Additionally, low metastatic melanoma can transform to become high metastatic and *vice versa*, indicating that stage progression is not directly linked to the metastatic potential. **Cecilia Bivik** showed that melanocyte

exposure to heat leads to an increase in HSP70 and promotes a resistance to UVB induced apoptosis. Resistance to UVB-induced apoptosis implicated a blockade in cathepsin D and cytochrome C release.

Session VI: Trafficking and transfer of melanosomes
Chair(s): Jo Lambert, Jose-Carlos Garcıa-Borron

The session on trafficking and transport of melanosomes included three invited lectures and three oral presentations, dealing with various aspects of melanosome biogenesis, motility and transfer in mammals and non-mammalian model systems. The first lecture of the session was delivered by **Bernhard Wehrle-Haller**, who described new data on the dimerization and the intracellular transport of the Kit-ligand (Kitl). This growth factor, also known as stem cell factor, binds to and activates the c-kit tyrosine kinase receptor expressed by melanocytes and is a key regulator of migration, proliferation and survival of melanocyte precursors and adult melanocytes. Although the receptor binding domain of the Kitl is known to consist of a non-covalent dimer expressed as an integral membrane protein of the keratinocyte plasma membrane, the mechanism and subcellular localization of dimerization, as well as several details of the precursor's activation process are poorly understood. The authors used an elegant fluorescence complementation technique to gain further insight on the dimerization process. For this purpose, Kitl constructs fused with either the N-terminal portion or the C-terminal domain of a yellow fluorescent protein were co-expressed, and dimer formation was followed by assessing the fluorescence signal. Using this approach, evidence was obtained pointing to the involvement of the transmembrane fragment of Kitl in dimerization, a process that would take place in the endoplasmic reticulum and would require the presence of two intact and functional export motifs. **Miguel Seabra** reviewed the role of Rab GTPases in the regulation of melanosoma biogenesis and motility, with emphasis on Rab38 and Rab27. The chocolate (*cht*) mutation in the mouse results from a loss-of-function mutation in Rab38 (G19V), but in the *cht* mouse the related Rab32 GTPase partially compensates this defect. Accordingly, depletion of Rab32 in *cht* melanocytes by means of siRNA technology leads to a much higher loss of pigmentation, with a retention of melanosomal enzymes in the Trans-Golgi region. This shows that Rab38 and Rab32 play a key role in early steps of the traffic of melanosomal enzymes. On the other hand, Rab27a may participate in latter steps of melanosoma maturation, necessary for normal transfer of the organelle. This is performed by interaction with related but not identical proteins in the skin and the RPE, where Rab27a apparently recruits Myrip and Myosin VIIa. Therefore, different Rab GTPases act sequentially to regulate the acquisition of a full and functional complement of melanogenic proteins during melanosome biogenesis, as well as on organelle movement and probably transfer. **Julio Valencia**, from Vince Hearing's laboratory, presented new and relevant results on the effects of glycosylation on the intracellular traffic and transfer of Pmel17/gp100. The pathway of Pmel17/gp100 maturation and transport to the melanosome is particularly complex, and the subject of intense scrutiny, partly because Pmel17/gp100 has several unusual features such as the proteolytic processing, the possibility that it may be partially secreted by melanocytes, and the possible occurrence of a sorting pathway involving the plasma membrane. A new antibody directed against the core region of Pmel17 (αPEP25h) has been obtained. This highly specific probe allowed to demonstrate differences in the glycosylation pattern of mature and immature Pmel17, as well as an unusual cytoplasmic staining of melanocytes and melanoma cells. Moreover, αPEP25h specifically stained the extracellular spaces between keratinocytes, suggesting a role in cellular communication. Therefore, it would appear the Pmel17 may serve other functions in addition to its central contribution to the formation of the melanosomal matrix.

Session VII: Human pigmentary disorders I : vitiligo
Chairs: Prof. Agustin Alomar, Prof. Silvia Moretti

Prof. Alan Taieb (Bordeaux, France) presented a lecture on "Definition and assessment of vitiligo: a progress report of the Vitiligo European Task Force". The lecture summarizes the work done by the Vitiligo European Task Force (VETF) to assess vitiligo and treatment outcomes using a system which combines analysis of extent, stage of disease (staging), and disease progression (spreading). After the system had been tested at several European institutions and discussed at three previous workshops, it was validated at an additional workshop with patients. This system could be easily handled in clinical practice, although a training to decrease inter-observer variability is advisable. Further steps will include guide lines for therapeutic indications and prognosis, large tests for clinical trials, and a teaching tool for scoring vitiligo on web sites such as ESPCR or EADV sites. **Dr. Mauro Picardo (Rome, Italy**) presented a lecture about "New trends in vitiligo research", which synthesizes the recent studies about vitiligo pathogenesis. The role of autoimmunity, with the occurrence of anti-melanocyte specific antibodies and melanocyte-specific T cell clones, is underlined, but more emphasis is given to a primitive, non immunologic, melanocyte damage. This damage seems to involve deregulation of c-kit-MC1R/p38-ERK-CREB/MITF axis, and appears to be related to an increased release of toxic by-products, able to inhibit the c-kit/kinases/MITF pathway. The cellular impairment involves also an initial lipidic defective arrangement in the membrane bilayer, possibly leading to exposure of new antigens. **Prof. Agustin Alomar (Barcelona, Spain)** talked about "308 nm excimer laser in the repigmentation of vitiligo", giving informations about the mechanism of action of this therapy which is still poorly understood. The authors conducted an immunohistochemical study, taking biopsies from perifollicular areas of vitiligo patches before and after treatment. An increased expression of Melan-A, c-kit and Bcl-2 molecules was observed in the basal layer after treatment, in association with the repigmentation process. **Prof. Silvia Moretti (Firenze, Italy**), spoke about "PAR-2 expression in vitiligo and halo nevus", concerning the evaluation of the protease-activated receptor (PAR)-2, expressed on keratinocytes and involved in epidermal pigmentation, by immunohistochemistry. PAR-2 protein was

expressed on the epidermis of lesional and perilesional vitiligo skin, with a weaker staining in lesional skin, whereas normal control epidermis was more intensely stained. Halo nevi showed a strong reaction on nevus cells, while overlying epidermis showed a weak staining; common nevi revealed a good reactivity on both nevus cells and overlying epidermis. These data confirm that PAR-2 is involved in the pigmentation process and its down-regulation may be associated with pigment loss from the epidermis. **Dr. Jasper G. van den Boorn (Amsterdam, The Netherland**), talked about "Vitiligo-infiltrating T cells kill autologous melanocytes within the skin tissue", providing additional evidence for the autoimmune hypothesis of the pathogenesis of vitiligo vulgaris. The authors had developed an ex vivo model of human autoimmune disease, with which HLA-A2/tyrosinase-specific T cells were shown to be able to kill melanocytes in non-lesional skin of vitiligo patients. Now the functional activity of the autologous T cells found in perilesional vitiligo skin was investigated: these lymphocytes could infiltrate autologous non-lesional skin explants and cause apoptosis of epidermal cells, including melanocytes, and cytotoxicity appeared mainly mediated by CD8+ T cells.

Session VIII: Human Pigmentary disorders II: albinism
Chairs: Richard King, Yasushi Tomita

Session IX: Melanoma and nevi
Chairs: Dorothy Bennett, Robert Ballotti

This morning plenary session started with the presentation of **Anja Bosserhoff**, who reported interesting connections between some common alterations in melanoma progression. It was known that upregulation of Snail, in melanoma, induced a loss of E-cadherin, which mediates interactions with surrounding keratinocytes. Anja Bosserhoff showed that the loss of E-cadherin is responsible for the activation of NFκB frequently observed in melanoma. This sustained NFκB activity might be involved in the impairment of the apoptotic response, thereby favouring melanoma development. Further, NFκB seems to stimulate the N-cadherin promoter, while E-cadherin re-expression in melanoma inhibits the N-cadherin promoter. Thus the loss of E-cadherin would lead to the upregulation of N-cadherin expression allowing melanoma cells to interact with dermal instead of epidermal cells. **Juan Recio** used the Mt-HGF transgenic mouse model and a proteomic approach to find new molecules within the HGF pathway, involved in melanoma development. This screening led to the identification of MO25 (Cab39) as a component of the HGF signalling pathway. MO25 belongs to a tripartite complex together with Strad and LKB1/STK11 - the protein kinase and tumor suppressor mutated in Peutz-Jeghers syndrome. Further, HGF was shown to induce phosphorylation of LKB1 at Ser428, through activation of the ERK/p90RSK pathway. Interestingly, some LKB1 substrates such as MARK3, MARK4 and QSK seem to be upregulated during melanoma progression. Taken together these results link HGF signalling as well as oncogenic activation of the ERK pathway (by *NRAS* or *BRAF* mutation) to LKB1, making LKB1 a potential target for melanoma treatment. **Celia Badenas** presented a study on genetics of melanoma and nevus predisposition in a Spanish population. Here, only 20% of families with 2 or more melanoma cases and 16% of patients with multiple primary melanomas have a mutation in *CDKN2A*. Further, mutation in this gene was observed in only 2.3% of sporadic cases. Melanomas in patients bearing a *CDKN2A* mutation appear earlier (mean 36 yr versus 50 yr). Although a 9p21 locus has been associated with dysplastic nevus syndrome, mutations in *CDKN2A* (located in this region) do not always lead to nevus susceptibility. Celia Badenas described a candidate gene in this region (PLAP/*PLAA* – phospholipase A2 activating protein), with polymorphisms related to an increased risk of developing nevi. By studying epigenetic regulation in melanoma, **Daniela Kovacs** identified *RAB33A* as one of the genes upregulated upon treatment of melanoma cells with a demethylating agent (5-Aza-CdR), which inhibits growth. The role of RAB33A in melanoma remains puzzling. However, among the nine available studies designed to identify melanoma-specific dysregulated genes, RAB33A is the only gene that has been identified in 4 studies as being downregulated, indicating that RAB33A silencing might play a role in melanoma development. **Lionel Larue** presented a new mouse model expressing a stabilized, nuclear -catenin in the melanocyte lineage. ß-catenin shows activating mutations in 3% of melanomas and has a nuclear localisation in 30% of melanomas, but its role in melanoma is not fully understood. The transgenic mice presented here were grey with a white belly, while the wild type mice were black. This was explained by an inhibition of melanoblast growth and migration by activated -catenin. However, -catenin favours immortalization, by silencing $p16^{Ink4a}$ expression, although it does not induce transformation. These results provide a molecular link between -catenin and the melanoma susceptibility gene *CDKN2A* encoding $p16^{Ink4a}$ and could explain the role of -catenin in melanoma development. Immunotherapeutic approaches were the subject of the presentation by **Damià Tormo**. In C57BL/6 mice, genetic immunization with recombinant adenovirus encoding human DCT and activation of the innate immune system via peritumoral injections of Toll-like receptors ligands induced rejection of syngeneic B16 melanoma implanted in the skin. In Mt-HGF x $CDK4^{R24C}$ transgenic mice, which develop multiple invasive melanomas following neonatal carcinogen treatment, the same vaccination protocol resulted in delayed growth of autochthonous primary and metastatic melanomas but did not induce tumour regression. These mice bearing multiple autochthonous melanomas did not reject B16 melanoma despite the use of the same vaccination protocol, suggesting the development of tumour immunotolerance.

Session X: Clinical aspects of melanoma
Chairs: Dr Inger Rosdahl, Dr Josep Malvehy

Melanoma clinica, genetic susceptibility and environmental factors
Contributed by Dr Susana Puig
We had two invited speakers and first Dr Susana Puig from the Melanoma Unit at the Dermatology Department in Hospital Clinic of Barcelona highlighted melanoma as a heterogeneous and multifactorial disease where susceptibility genes interact with environmental factors. She emphasize that there are several roads to melanoma and presents emerging evidence of divergent pathways leading to melanoma, each involving diverse patterns of sun exposure and distinct genetic changes. She described the variation in genotype, phenotype and risk-behaviour in the development of different melanoma subtypes. Individuals with an instable melanocyte system with increased proliferation of melanocytes and with many nevi are at risk to develop superficially spreading melanoma on intermittently exposed skin areas. High naevus counts have also been found to be more predictive of melanoma risk in younger than older subjects. Somatic genetic alterations commonly found in melanoma tissue from the superficially spreading type of melanoma are UV signature mutations in the BRAF/NRAS-gene. This signature mutations seems to be significantly influenced by other genes such as melanocortin receptor gene. In contrast, melanomas occurring in older ages in skin with chronic sun damage are negatively correlated with nevus number. These melanomas, in contrast to nevus-associated melanomas, do not show mutation in BRAF/NRAS in most of cases, but frequently have an aberrant expression of p53 and cyklin D1. A today unknown but third pathway might be involved in the development of acral and mucosal melanomas which seems to arise independent of sun exposure. Dr Puig concluded that this wide variation in etiology and formation and biology of malignant melanoma must have a consequence for the design of future preventive programs, early diagnosis and therapy.

Current experimental therapies and future directions in metastatic melanoma,
Contributed by Dr Ghanem Ghanem
The second invited speaker at this session was Dr Ghanem Ghanem who presented an extensive over-view of ongoing research and clinical trails for treatment of metastatic and disseminated malignant melanoma. More than 200 trails are conducted of which more than 55% are immunotherapy. Despite intensive research the prognosis of advanced melanoma is poor with about 50% 5 year survival of patients with limited regional lymph nodes involved and less than 5% in stage IV patients with distant metastasis. Dr Ghanem exemplified in his presentation the most promising results by stimulation of immunotherapy particularly with GM-CFS, Toll-like receptor 7 agonists and anti-CTLA-4mAb. Trails with various combinations of chemotherapeutic drugs and new potentially interesting techniques for targeting drug delivery comprise 22% of the studies. Another 14 % of the trails include molecules directed against specific targets and signaling pathways as VEGF-R tyrosine kinase, PKC, cdk and mTOR inhibitors followed by inhibitors of proteosomes, topoisomeras, histonedeacetylase, HSP among others. Several of these studies report improvement of disease and others valid but minor encouraging results. Following an overview of the response of these new experimental drugs Dr Ghanem conclude that the key to successful treatment is to design the right combination and dose of drugs for each tumor and personalize treatment based on tumor as well as patient markers.

Vitiligo development in metastatic melanoma patients following vaccination with autologous GM-CSF_transduced tumor cells
Contributed by Dr Rosalie Luiten
The first of the two oral presentations chosen by the chairs was given by Rosalie Luiten from Department of Dermatology, University of Amsterdam, who presents results from a phase I/II vaccination study in stage IV melanoma patients. Sixty-four patients were randomized to a high or low dose vaccination regime at three weeks interval. Vaccine preparation with autologous tumour cells retrovirally transduced with the GM-CFS was successful in 56/64, but due to rapid progression of the disease only 28 subjects finished the vaccination program, 14 of them received the high dose vaccine regime. The T-cell activation against melanoma antigen was monitored for MART-1, tyrosinase, gb100, MAGE A1 and A3 using HLA/peptide tetramers and functional assays. All patients tolerated the vaccine well and six of the patients randomized to the high dose regime had a disease free survival of more than 5 years. 3/14 patients getting the high dose vaccine formed MART-1 and gb100 specific T cells in the blood during vaccination. Two of them developed vitiligo on several body sites and in those MART-1 and gb100-specific T-cells infiltrated the vitiligo skin. This raises the question concerning vitiligo and possibly the presence of active T cells infiltration in vitiligo skin areas as a possible prognostic marker in melanoma. In spite of the limitation in the response and survival of the study these therapeutic strategy may lead to better results in stage IV patients are worth further investigations.

Incorporation to melanoma cells and induction of cell death by Magnetite-Conjugated N-Propionyl Cystaminylphenol (NPRCAP/M) nanoparticles for melanoma CTI therapy.
Contributed by Makito Sato PHh D student
This oral presentation of Makito Sato from Sapporo Medical University describes a the experience of potentially interesting melanoma therapy selectively targeting melanoma cells, reacting with tyrosinase causing melanoma cell death. NPRCAP was conjugated to magnetite nanoparticles and in cell culture systems it was demonstrated using electron microscopy that NPRCAP/M was selectively incorporated in large clusters in melanoma cells. In contrast such nanoparticles were aggregated extracellularly around the cell surface in non-melanoma cells. Alternating magnetic field fragmented the

melanoma cells exposed to NPRCAP/M. By flow cytometry and agarose gel electrophoresis it was shown that the melanoma cell death was due to necrosis and not to apoptosis. Some promising results from in vivo mouse studies with NPRCAP/M and alternating magnetic field treatment were presented. The presentation was followed by several questions from the auditory. According to professor Jimbow who is the senior investigator in these studies NPRCAP is a non-toxic substance and treatment trails has just been started in humans.

Session XI: UV-light and Epidermal Biology
Chairs: Dr VJ Hearing, Dr L Marrot
Contributed by Drs VJ Hearing and L Marrot

This session focused on the role of UV in determining skin function, primarily via stress and MC1R responses. The session began with an invited lecture from **Markus Böhm**. He reviewed the field of stresses on the skin, our primary defense against stresses from the environment (e.g. UV) and from intrinsic sources (e.g. OXPHO) and endogenous sources (e.g. LPS). Responses to such stress are varied and can involve neuropeptides (e.g. melanocortins such as POMC and αMSH) induced in human keratinocytes by UV. They have examined this by exposing human skin to SSR (at 2 MED), and then taking suction blister biopsies at 3, 6 and 24 hr after that. They characterized up-regulation of POMC RNA expression and MSH that was detectable in blister fluids after UV radiation. Prior application of sunscreens could prevent that response. In culture, H_2O_2 (at 500 μM, the physiological level) reduced levels of mRNAs encoding POMC and MC1R in normal human melanocytes but it induced those mRNAs in keratinocytes. He pointed out that whether this pattern occurs in situ needs to be determined in the future. He noted that a number of studies have shown now that MSH protects melanocytes from UVB-induced apoptosis, and he finds that this protection also occurs for keratinocytes in a similar manner. MSH also reduces production of DNA damage (in the form of cyclobutane pyrimidine dimers) in UVB-irradiated melanocytes (and in dermal fibroblasts), but had no effect in XPA-deficient fibroblasts proving the nucleotide excision repair (NER) is involved. In response to the audience, Markus Böhm mentioned that assessment of the role of p53 in this process was in progress. The next invited lecture was given by **Zalfa Abdel-Malek**. She reviewed the 3 types of DNA damage that result from UV exposure, and noted that there were 3 possible outcomes of such damage: (1) growth arrest/DNA repair; (2) apoptosis, or (3) survival and possible heritable mutagenesis. In human melanocytes, eumelanin correlates directly with visible pigmentation, pheomelanin varies. She reviewed MC1R function and the role of agouti signal protein (ASP) in regulating eu- versus pheo-melanogenesis. There are several critical mutations of MC1R that dramatically affect its function. Loss of function MC1R mutant melanocytes have higher levels of DNA photoproducts after UV exposure and have decreased levels of NER. Levels of NER can be stimulated by MSH if a functional MC1R is present. She then reported the time course of responses to UV in the presence or absence of MSH as assessed by DNA μarray technology. There are large changes in cells with functional MC1R, few changes otherwise. Genes involved with apoptosis, pigmentation, cell cycle and DNA repair are in general decreased by UV in the absence of MSH, underscoring the importance of MC1R function in the regulation of melanocyte function at many levels. Heme oxygenase 1 expression was stimulated by MSH and MSH+UV, suggesting an involvement of MSH in endogenous antioxidant defenses. The final invited lecture in this Session was given by **Marie-Dominique Galibert**. She reviewed the skin as the first defense against UV radiation, and the role of pigmentation in protection against UV damage. She summarized factors secreted by keratinocytes that influence melanocyte function. Her lecture focused on the transcription factor USF-1, which is a stress response transcription factor that is involved in regulating pigmentation. USF-1 knockout mice have normal pigmentation, and have comparable levels of expression of MC1R, tyrosinase and other pigment-related genes. However, increases in POMC in response to UVB are lost in USF-1 knockout skin. As a model, they use in vivo SSR-radiated skin biopsies. They took biopsies 5 hr about UV radiation. All pigment genes examined were increased but USF-1 levels were not affected. She described a new isoform of USF-1 (termed M-USF-1) that is expressed after various types of stress; normal processing of USF-1 includes at least one phosphorylation event. The phosphorylated form of USF-1 stimulated pigment gene expression (via its binding and activation at the E-box) but the M-USF-1 had no effect. Future work will characterize the role of the various forms of USF-1 in regulating pigmentation. There were then 3 lectures presented from the abstracts submitted to this topic. **Dr. Santos Alonso** presented work on the gene expression profiling of human melanocytes after chronic UVA+UVB radiation. They examined the responses of lightly pigmented and darkly pigment melanocytes responding to 50 mJ/cm^2 once per day for 6 days. They found that Tyr, Tyrp1, Dct, GPCR143, MART1, OA1 and Pmel17 are highly expressed by all types of normal human melanocytes (regardless of constitutive pigmentation) but that those genes are not regulated by UV. This was a bit surprising and there was some comment from the audience that perhaps the dose was too high and/or this was the wrong time-point to examine, since UV-induced regulation of those genes would normally be expected to occur. **Eugene Healy** then discussed the MC1R regulation of pigmentation and its role in skin cancer. He summarized how MC1R functions in vivo and used hairless mice as a model for his UV studies, mice that had black, albino or yellow (e/e) backgrounds to affect the type of pigmentation they produced. They found no difference in background levels of DNA damage (measured as CPD) in the absence of UV (as expected) but even after 6 weeks of SSR treatment, they saw no differences in CPD levels according to the pigmentation (It was however underlined by the audience that CPD global repair was low in mice and clearly not as efficient as in humans). However, there were differences in protection against mutations in p53. They conclude that pigmentary and non-pigmentary mechanisms are involved in these responses. The final talk in the Session was by **Barbara Bellei**, who reviewed the importance of β-catenin in the melanocyte lineage during development and also in adult tissues. She reminded everyone of the dual nature of β-catenin function depending on its subcellular localization.

Expression of β-catenin was decreased after exposure to UVA. She noted that GSK3β plays an important role in targeting β-catenin for degradation in proteasomes. UVA radiation decreased GSK3β which suggested that the decrease in β-catenin after UVA is GSK3β-independent. Rather, caspases (especially caspase-3) seem to be responsible for the decrease in β-catenin after UVA. She concluded that Wnt signaling plays a crucial role in the regulation of stress-induced mechanisms activated in normal human melanocytes by UVA.

Commentary: Unraveling the Melanosome

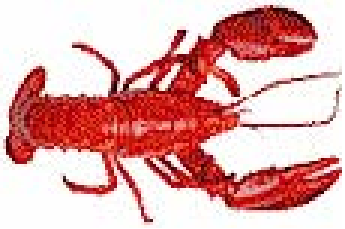
Vincent J. Hearing, PhD, Chief, Pigment Cell Biology Section, Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD (hearingv@nih.gov) November, 2006

Introduction

The story underlying how I became involved in studying pigmentation, and then developed that into my scientific career is a convoluted one built on a large number of coincidences. As background, I was born into and grew up in a military family and with my 4 younger siblings, moved on the average of every 1.5 years until I was 18. We lived in France, Germany, Hawaii, Kansas, Alabama, Virginia (3 times) and Washington DC (twice). I suppose that explains why I've taken roots in the Washington DC area and haven't moved my home base since then. It also probably explains why I love to travel and visit other cultures/countries at every opportunity. To me, the chance to travel to scientific meetings around the world and collaborate with other scientists on a global basis is perhaps the most rewarding and surprising aspect of a scientific career.

Materials and Methods

To receive a B.S. degree in Biology at Georgetown University (at least back then) you needed to do a 1 year senior science research project. That was my introduction to the scientific method. At the time, among the faculty there, Dr. Sanford Vernick was an energetic young Professor who had a great sense of humor and who was willing to mentor me; he had only 1 drawback, i.e. he was an ichthyologist, and to work with him I had to take on a project in that field. He interested me in learning and then using electron microscopy (then a relatively new technique) to study the blood cells of lobsters; it turns out their blood is green because of the pigment hemocyanin and I also found that they were quite delicious once the blood had been collected each Friday at lunchtime. We managed to publish a scientific paper on that study which introduced me to the publication process and to the rewards and challenges there (1). Following graduation, I enrolled at The Catholic University of America in a Ph.D. program majoring in Cell & Molecular Biology (a field also growing rapidly back then). My mentor was Prof. John O'Brien, a celebrated botanist, but he also required me to perform my dissertation research in his field, which was an abrupt switch in direction. He interested me in studying the mechanism by which plants grown in the dark are unpigmented but then rapidly begin to produce the pigment chlorophyll and turn green when exposed to sunlight. Again, it was a fascinating study that involved pigment, albeit pigment in organelles (chloroplasts) but not yet of the mammalian variety (2). That final step arrived the following year when I met a gorgeous young Chemistry student and it became quite clear that I would need to improve every aspect of my dubious character to attract her and also that I needed a source of additional income to support us if I was successful in doing that. Back in those days, the stipend for teaching graduate students was about $3,000 (a year, not a month), but it was tax-free and one person, but not two, could survive on that. The National Institutes of Health was close by and the Dermatology Branch there was recruiting a part-time technician to run their EM facility. I applied for that position and was successful. The pet project of Dr. Marvin Lutzner, the Chief of Dermatology, was the Chediak-Higashi syndrome and he wanted me to do the ultrastructural analysis of the mouse model for that disease - the beige mouse. While we were at it, we decided that we might as well look at the other pigment mutants available from the NIH mouse colony, which included albino, pink, brown, dilute and a few other types of mice that have since become famous.

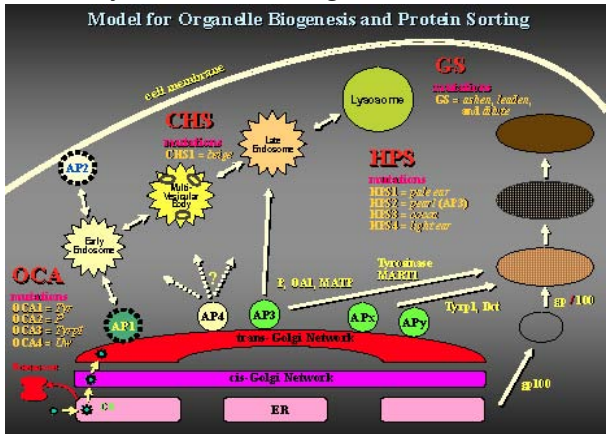


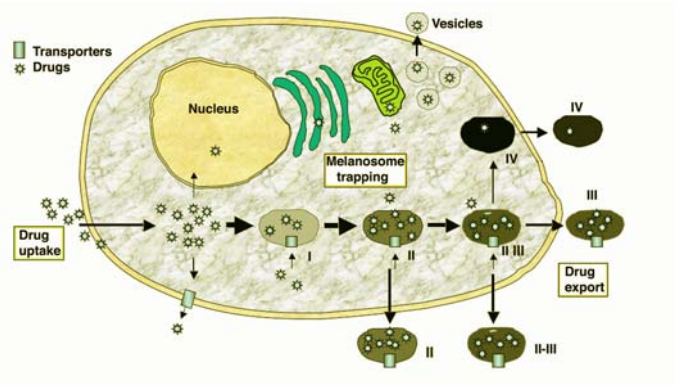
We even cross-bred many of those mutants to look at the effects of various combinations. So my days were spent during daylight hours studying plant pigmentation at Catholic University and much of the night studying mammalian pigmentation at NIH. Not only did both of those projects turn out successfully (3), but my pursuit of Betsy Brown was equally successful and we were married during my sophomore year. Although I had a few offers for post-doctoral studies, I decided to accept the one offered by the National Cancer Institute because I was now hopelessly entranced with the process of melanogenesis, and, given the tremendous resources at NIH, I felt that was THE place to be, and a foot in the door as a post-doc would be a good start. Of course, it worked out that way and within a few years I converted to a Staff Fellow here, and a few years after that was given tenure and became a Principal Investigator. Back in those days, we were each allowed to have one post-doctoral fellow and one clinical associate. Prof. Makoto Seiji contacted me to ask if I would accept Dr. Yasushi Tomita from his group as my first foreign post-doc; given Seiji's reputation in the field I was quick to agree and Tomita (who has now progressed to Prof. and Chair of Dermatology at Nagoya University) became my first post-doc. Seiji hosted the IPCC at that time and died soon thereafter, but fortunately, Prof. Yoshiaki Hori took over the role of sending me outstanding post-docs from his University, which included Koichiro Kameyama, Katsuhiko Tsukamoto, Kazunori Urabe

and Minao Furumura. The restrictions of space in a small laboratory and having only one post-doc at a time was impeding progress in a time filled with explosive advances in techniques and understanding of regulatory processes at the subcellular level. Just about that time, Marvin Lutzner decided to move on and the new Chief was Steve Katz, whose specialty was the immune functions of the skin, and melanocyte biology was no longer an emphasis of that group. Fortunately, Dr. Lloyd Law, who had discovered the combined chemotherapy approach to treating leukemia and was thus a celebrity at NCI, was very interested in studying the immune responses to melanoma (and why they aren't very successful). He recruited me to join the Laboratory of Cell Biology with the promise of more space (always a major problem at NIH) and better resource support. He did that (and more) to advance my career, really pushing me to design fail-safe experiments with all appropriate controls that ask the most critical questions. He piqued my interest in melanoma biology and I piqued his in the regulation of pigmentation, so we had an excellent collaborative spirit in our laboratories. With more space and resources, I was able to gradually expand my research group from 2 to its current level of 9; this allowed me to take outstanding post-docs not only from Japan, but also from Europe and other Asian countries as well as from the Americas. That list is very long now, but includes >60 names, and I am very proud that most of them are still actively involved in clinical or basic research.

Results

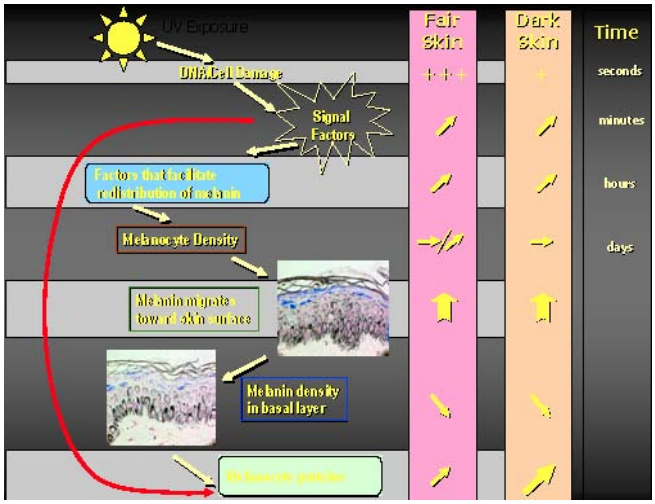
The evolution of my research interests thus went from invertebrate pigmentation (hemocyanins) to plant pigmentation (chlorophyll) to mammalian pigmentation (melanins). As noted above, our studies on melanogenesis began with ultrastructural and enzymatic analysis of pigment cells in mammals and amphibians and, aside from the fascinating process of their maturation from premelanosomes to melanosomes (as first described by Seiji), the fact that active tyrosinase could be detected in the Golgi apparatus and in coated vesicles en route to melanosomes, but was not normally active in those organelles was just too much of a challenge to resist. Back then, only tyrosinase was known as an important melanosomal protein, but later the issue became even more complex as other enzymatic and structural components of melanosomes were discovered (Tyrp1, Dct, Pmel17, etc). One then had to consider whether they were trafficked together to melanosomes or were segregated in different vesicles and were only brought together at melanosomes (which might explain the delay in melanin synthesis). As we are all aware, tremendous strides have been made in defining all of this (and pigmentary diseases that result when the process goes awry) but not all questions have been resolved about the trafficking of melanosomal proteins, or even about the functions of some melanocyte-specific proteins (e.g. P, MATP and OA1) and how they regulate that process. Proteomic analysis of different stages of melanosomes has revealed the incredible number of components required to make melanosomes and to push them through their maturation process (4;5). In sum, much remains to be done to fully characterize the melanosome and I don't foresee a time in my career when all interesting questions on this topic have been resolved and I'll need to look around for something else to work on.





When I transferred my research group to the Laboratory of Cell Biology, Dr. Law followed through on his promise to expand my space and resources, so in turn I expanded my research effort into studying melanoma immunology with him. We soon found that one could relatively easily immunize mice against subsequent challenge by tumor cells (either subcutaneous or intravenous to look at effects on primary tumor growth or 'metastasis', respectively). In fact, melanoma vaccines prepared from any one type of mouse melanoma (except S91) was able to protect against the others (6). The problem came when trying to treat existing tumor burden and, although we were able to get some prolonged survival of tumor-bearing mice using a number of approaches, none could be said to be effective in eradicating the tumors, something that is unfortunately also true in the clinic with human melanomas. Dr. Law retired about a decade later and his successor was Dr. Michael Gottesman, who had made his fortune by cloning and characterizing the first multi-drug transporter (MDR1), a pump that can effectively keep most chemotherapeutics out of tumor cells, thus allowing their survival. Even worse, expression of MDR1 is often stimulated by drug treatment so the most drug-resistant cells persist and form an even more intractable tumor. Interestingly, a large number of MDR family genes have now been cloned (>40) and one of them, ABCB5, is expressed only in melanocytic cells. Of particular interest, while most MDR proteins are on the cell surface,

ABCB5 is localized intracellularly on the melanosome membrane (7). MDR proteins seem to have important functions in normal cells where expressed, which work to their disadvantage when they become transformed to malignant cells. We think that the normal function of ABCB5 may be to pump toxic intermediates (produced during melanin synthesis) to keep them within melanosomes and thus avoid toxicity to the cells (8). However, in melanoma cells, this pump would efficiently transport any drugs taken up into melanosomes, thus sequestering them and increasing the resistance of melanoma cells to chemotherapy (also a well known phenomenon in the clinic). As the regulation of melanin biosynthesis was gradually being clarified at various levels (by ours and many other laboratories), we began to address a critical basic question about the role(s) of melanins/melanosomes in photoprotection of the skin. This had been assumed for many years based on a large number of in vitro studies, but the actual mechanisms involved and whether eu- or pheo-melanin was to be preferred was not known about human skin in situ. This is of course of critical interest to the NCI with respect to photocarcinogenesis. We had been working on this question using mouse models, but the architecture of mouse skin which is so distinct from human skin with respect to melanocyte (and melanin) distribution had made such studies far from appropriate for physiological understanding. When we were unexpectedly approached by the FDA to see if we were interested in analyzing human skin specimens of varying racial/ethnic origin that were UV-irradiated in situ, it was the ultimate gift. We were able not only to look at DNA damage in human skin of varying phenotypes, but also to use those specimens to examine the basic physiology of melanocyte function in human skin. In recent years, we have published a number of studies that have provided important insights into the DNA damage that occurs in various types of cells in the skin, and which (in my view at least) provide interesting clues to the dramatic differences in incidence of all types of skin cancers in light as opposed to darkskin (9;10).



Discussion

Working at NIH has freed me from many time-consuming constraints, such as grant writing (yes, at NIH we have to write annual reports and we have Site Visits every 4 years, but I realize this is minimal effort compared to academia) and teaching (yes, we have some students and of course lab workers, but teaching lab techniques is a breeze compared with teaching courses). So while space has always been a critical issue here, budget and other resources have not been, although those have gradually eroded in recent years to become challenging here as well. Since I didn't have to spend excess time in those duties, I decided that I should make every effort to use the time saved towards promoting research in the field. This has taken the form of holding a large number of political offices, developing and distributing critical reagents, serving as editor on a number of books and our society's journal, and organizing meetings to facilitate interactions in the field. Beginning near the end of the -80's, this has turned into an almost comical sequence of events that has just now concluded:

- 1989 - Organizer, 2nd PASPCR Meeting - the formation of the PASPCR (led by Dick King and JimNordlund) in 1987 led to its first meeting in Minneapolis in 1988, but where would the 2nd more formal meeting occur? Thanks to my supportive boss, who helped ensure that NCI would underwrite most of the costs of that meeting, we could rapidly organize that meeting in Bethesda.
- 1991 - 1993 -PASPCR Council and President - probably due to name recognition as Organizer of the PASPCR Meeting, I was elected to the Council and then as President.
- 1993 - 1996 -IFPCS Council and then President -by virtue of being the PASPCR President, I was automatically on the IFPCS Council, by virtue of being on the IFPCS Council when the rotation dictated that the IFPCS President come from the PASPCR - voila!
- 2000 - Editor, Pigment Cell Research - by virtue of retiring as IFPCS President and thus beingavailable, at the time when the next Editor of PCR was to be selected from the PASPCR or ESPCR -voila!
- 2005 - Organizer, 19th IPCC - by virtue of retiring as Editor of PCR at the time when the IPCC was due to be hosted by the PASPCR - voila!

Not that there weren't duties and responsibilities, and financial requirements and support personnel required by all of those functions above, but there is no doubt that the resources of the NIH made that possible whereas had I not been at NIH, I would not have considered doing much, maybe all, of that.

It has been extremely rewarding to see that over the years, interest in pigment research at NIH has grown exponentially. When I first began working here, mine was the only basic research lab in this field at NIH, but over the past 15-20 years, many Institutes and other local government agencies have become involved in pigment research, many of them studying development, differentiation, dermatology, photobiology, etc. The support of those other groups and our underlying focus group (the NIH Pigment Cell Interest group) made the recent IPCC a rousing success at every level and a relative breeze to organize and fund. It is my hope that interest in pigment research will continue to grow and that NIH will recruit and expand laboratories studying these various important topics. Given past success by pigment groups here and the important

problems that remain, I think it is a safe bet to turn out that way.
My political and other outside duties now behind me, I am refocusing my efforts for the balance of my career to run my laboratory, to catch up on my writing and to help others get started. It seems a bit strange to have this extra time now but also shocking at how far behind in my scientific work I had gotten. I'm looking forward to at least trying to reach an equilibrium point before retiring from NIH.

Acknowledgements
Finally, there is no way to begin to adequately thank everyone for their support, but let me briefly try. First, my wife Betsy (who also grew up in a military family and thus shares my love of travel), who has a fantastic career of her own, for always putting that secondary to supporting and pushing me forward at all times. My children (Brian, Laura and David), who tolerated my odd sense of humor and dedication to work and somehow prospered despite that. My mentors, all through my schooling and career at NIH who gave me almost anything I asked for (or at least what I semi-deserved). Finally, my post-docs and collaborators over the years, who are now legion and who have enabled my scientific achievement every step of the way.

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CURRENT LITERATURE

1. Chemistry of Melanins and other Pigments

(Dr. A. Napolitano)

The structural and physical properties of natural and synthetic melanins have remained the main research focus in the last three months.
A most interesting review by Paul Meredith and Ted Sarna has appeared (*Pigment Cell Res*.) summarizing the massive body of literature of the last few years (but also older pioneering papers) trying to provide a critical analysis of the sometimes conflicting results. Aim of the authors was to draw the crucial link between the structural properties of eumelanin and its macroscopic properties ranging from the absorption/ fluorescence properties to the condensed phase electrical properties that open new perspectives in the exploitation of melanins or melanin-based polymers as innovative materials, till the antioxidant and photoprotective properties, the most relevant to the pigment biological role.
The picture which emerges is that, although several advances have been done in developing more informative approaches to the optical properties of eumelanin, interpretation of the data is still limited by the lack of a generally accepted and experimentally tested model of what is termed secondary structure of the eumelanin that is how the polymer or a collection of oligomers are three-dimensionally assembled. Such a critical issue in turn relies on knowledge of how the indole monomer units couple and the properties of the oxidized forms of the oligomer species. This latter aspect has been addressed by an integrated pulse radiolytic computational and chemical investigation of the oxidation chemistry of selected DHI dimers. (Pezzella et al *J. Am.Chem.Soc*.) Data concur in indicating extended quinone methides as the most stable tautomers for all biindolyl quinones investigated. Upon oxidation, the non-planar geometries of the 2,2', 2,4' and 2,7' dimers are converted to extended quinone methide structures in which the double bond constrains the biindolyl system to an approximately planar geometry, possibly more amenable to be assembled into a stacked layers polymeric architecture**.**
Another interesting outcome of this study is the discovery of significant differences between the oxidation behaviour of the dimers and DHI, cautioning the extension of concepts that apply strictly to the mode of coupling of DHI to higher oligomers.
The photoprotective properties of sepiomelanin toward blue light- induced apoptosis of RPE cells have been demonstrated and interpreted by EPR spectra as due to an increased intracellular melanin free radical concentrations.(Seagle et al. *Proc. Natl. Acad. Sci.*).

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2. Biology of pigment cells and pigmentary disorders

(Dr. M. Picardo)

A brief overview of the most recent papers on melanocyte biology and pigmentary disorders, focusing on vitiligo and melanoma.

The paper written by **Rouzaud** can be authoritatively placed between basic cell biology and photoprotection strategies. A new isoform (MC1R350) of MC1R is described by using histochemistry and molecular approaches (transfection and sequencing). It appears to be negatively involved in melanin synthesis regulation and MITF expression. The balance between MC1R317 and MC1R350 governs the amount of melanin. The presence in MC1R350 of five cysteine residues could account for its high stability and function. This isoform is mainly linked to darker skin pigmentation and photoprotection. **D'Orazio** provides new and relevant insights into the world of UV responses by using an animal model ($Mc1r^{e/e}$ mouse). Fair pigmentation inversely correlates with tanning response. In mouse mutant at MSH receptor (e/e) forskolin rescues eumelanin production indicating that (1) forskolin did not require ectopic SCF and (2) the loss of tanning response to UV could not be due to the irreversible inactivation of the melanogenesis pathway. Forskolin, by increasing cAMP, protects against UV-induced chronic keratinocyte damage. The study could represent the first step in the use of cAMP agonist for the topical rescue of UV-protection.
The crucial role of SCF/c-kit pair is supported by data provided by **Kang** who, evaluating melasma lesions using immunohistochemestry and RT-PCR, indicates an increased expression of SCF in lesional dermis and of
c-kit in lesional epidermis (the mRNA for both proteins is increased in lesional skin). This is in agreement with the recent insights supporting the role of SCF/c-kit defect in vitiligo. The **Schallreuter** group further focuses on the tetrahydrobiopterin metabolism in the epidermal cells (both melanocytes and keratinocytes) and evaluates the activity of some enzymes in the BH4 synthesis. Even if the results need further investigation, the data could represent additional information in the relationship between melanin and catecholamine pathways in healthy and pathological conditions. Western blotting analysis, RNA interference, and the analysis of some cellular and mitochondrial functional parameters provide the technical support for the evaluation of the apoptotic pathways in normal melanocytes and the kinetic sequence of the death process. **Liu**, author of the summarized paper above, furnishes interesting and useful data in melanocyte biology helping in the understanding of the physiological and pathological responses of the melanocytes to the stressors. The author indicates early data that suggest a crucial role for survivin in melanoma resistance to apoptosis.
Study of melanocyte stem cells is increasing and it could become the basis for understanding some pigmentation disorders,such as hair grey[illegible] and vitiligo. In agreement with that, **Arck's** manuscript is very interesting. She uses cultured hair follicles and, by combinating cellular and molecular approaches, demonstrates the specific susceptibility to oxidative stress of hair greying follicles, supported by the occurrence of mitochondrial DNA deletion and apoptosis. The authors underlined the relevant role of SCF/c-kit and Bcl-2 in the melanocyte defect leading to greying process. Considering the possible future relevance of melanocyte stem cells, **Na** provides useful information for the isolation and culture of the hair follicle and **Kauser** focuses on the link between hair follicle pigmentary unit and corticotropin-related peptides. The last paper by **Goding** is a clear, precise and exciting overview of the melanocyte world that establishes relevant links between the different approaches and knowledge.
The excellent paper from **Abdel-Malek** group published by Faseb J focuses on the possible clinical relevance of tetrapeptide α-MSH analogs in melanoma. α -MSH is part of the paracrine/autocrine network activated by UVB and capable of inducing the apt response of melanocytes to sun exposure. α -MSH via cAMP allows UVB-induced G1 arrest to be overcome and it is able to enhance DNA repair, compromised in melanoma, lowering the ROS production. The synthetic MC1R agonists could thus play a relevant role in the prevention of melanocyte neoplastic degeneration. Overall, this represents the biological basis for the above mentioned paper where, through an elegant and easy experimental approach (tyrosinase activity, proliferation, apoptosis, ROS generation), the authors evaluated the ability of some synthetic analogs to reduce the DNA damage induced by UVR, opening a new and interesting scenario in cancer prevention based on topical drugs. EGF has been reported to be involved in melanoma growth through RAS and BRAF. **Okamoto** evaluates the relevance of the A61G variant in melanoma history. He indicates that the EGF A61G variant could be taken as a parameter for melanoma relapse.
The immune involvement in the pathogenesis of vitiligo is strongly supported by several clinical and experimental evidence even if the the precise role still lacks a clear definition. **Lambe** and coworkers provide another fragment to the central and peripheric tolerance puzzle and its breakdown in vitiligo, unerlying the significance of Fas-Fas Ligand pathway in melanocyte death. The genetic background of vitiligo was investigated by **Gavalas**, who studies catalase polymorphisms, and by **Kingo**, who analyses MYG1 expression.

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3. MSH, MCH, other hormones, differentiation

(Dr. R. Morandini)

It is well know that alpha-MSH has pleiotropic effects. One (perhaps) of the most surprising is its possible antimicrobial properties (Catania, 2006). alpha-MSH was discovered to have antimicrobial activity against two representative pathogens: Staphylococcus aureus and Candida albicans. The antimicrobial influences of alpha-MSH appeared to be mediated by intracellular cAMP increase. The latter interferes with the yeast's own regulatory mechanisms of this essential signaling pathway. When considering that most of the natural antimicrobial peptides enhance the local inflammatory reaction, the antiinflammatory and antipyretic effects of alpha-MSH confer unique properties to this molecule as compared to other natural antimicrobial molecules. Synthetic derivatives, chemically stable and resistant to enzymatic degradation, could form the basis for novel therapies that combine antiinflammatory and antimicrobial properties. Another paper of Catania in the "Journal of leukocyte biology" focus on the melanocortin system in leukocyte biology. Leukocytes are a source of melanocortins and a major targets for these molecules. MC1R is the main receptor in leukocytes and other cells involved in immune reactions. The number of MC1R in leukocyte is lower than in melanocytes but the receptor activation needs picomolar concentrations while in melanocytes it requires nanomolar. The anti-inflammatory effects of MSH is mediated by the C-terminus (11-13) Lys-Pro-Val. It appears that MSH exerts its anti-inflammatory effect through CD14 and CD86 receptors. Other reported effects are: inhibition of NOS2 mRNA, inhibition of neutrophil migration, downregulation of IL8-R,… Immunosuppressive effects of alpha-MSH were independent of MC1R gene status. All of these data may lead to the design of very efficient antiinflammatory new molecules.
Schioth has made a very interesting study of some analogs of the C-terminal tripeptide of the alpha-MSH (alpha-MSH11-13). The author found that some of the new tetrapeptides have novel properties and act via MC-ergic pathways and also carry the anti-inflammatory alpha-MSH11-13 message sequence.

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4. Photobiology

(Dr. N. Smit)

Since MSH is known to play a role in the repair of UV induced DNA damage it is a logical consequence that a strategy is now developed to make use of various MSH analogs to be studied for their potential to enhance DNA repair and reduce DNA damage in the melanocyte and prevent melanoma. First studies in this direction are described by Abdel-Malek et al. Another approach is described by Kobayashi who used a gamma-tocopherol derivative that acts as an antioxidant, antiinflammatory and antipigmentation agent. Also the experiments by Larsson et al indicate that alpha-tocopherol may be protective for melanocytes.
The paper by Boscoe et al describes the effect of UV exposure on many different cancer types. Since vitamin D production is considered to inhibit tumor development a reduction in UV exposure may be responsible for a higher incidence of various cancer types. A positive association between solar UV-B exposure and cancer mortality was also found for several cancer types, e.g. melanoma. Scelo et al have investigated the risk of different types of cancer in association with ocular melanoma. This paper indicates that patients with ocular melanoma have also increased risk of cutaneous melanoma and some other cancer types. De Fabo describes some studies on the balance between the healthy effects of sunlight exposure and the deleterious effects finally resulting in melanoma.

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  Solar ultraviolet-B exposure and cancer incidence and mortality in the United States, 1993-2002. BMC.Cancer 6:264, 2006.
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  Hypothesis: hair cover can protect against invasive melanoma on the head and neck (Australia). Cancer Causes Control 2006, 17:1263-1266, 2006.

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Polymorphisms in the MTHFR and VDR genes and skin cancer risk. Carcinogenesis 2006.
This study suggests a possible role of the polymorphisms in methylenetetrahydrofolate reductase (MTHFR) and the vitamin D receptor (VDR) gene interacting with dietary intakes of folate and vitamin D in skin cancer development, especially for SCC.

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[UVB-induced skin damage and the protection/treatment--effects of a novel, hydrophilic gamma-tocopherol derivative]. Yakugaku Zasshi 126:677-693, 2006.

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Does sunlight have a beneficial influence on certain cancers ? Prog.Biophys.Mol.Biol. 92:132-139, 2006.

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Ultraviolet (UV)A- and UVB-induced redox alterations and activation of nuclear factor-kappaB in human melanocytes-protective effects of alpha-tocopherol. Br.J.Dermatol. 155:292-300, 2006.
CONCLUSIONS: UVA and UVB irradiation affected the intracellular redox state and increased the frequency of apoptosis in human melanocytes in vitro. alpha-Tocopherol might be a useful substance in protecting melanocytes from UV-induced damage.

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Basic Fibroblast Growth Factor Promotes Melanocyte Migration via Increased Expression of p125FAK on Melanocytes. Acta Derm.Venereol. 86:498-502, 2006.
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Human skin responses to UV radiation: pigment in the upper epidermis protects against DNA damage in the lower epidermis and facilitates apoptosis. FASEB J. 2006, 20:1486-1488, 2006.

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5. Neuromelanins

(Prof. M. d'Ischia)

The present commentary will focus mainly on a paper by Bush et al. entitled "The surface oxidation potential of human neuromelanin reveals a spherical architecture with a pheomelanin core and a eumelanin surface" (Proceedings Natl. Acad. Sci. (USA) (2006), 103(40), 14785-14789)
This paper describes the results of an investigation of neuromelanin from human substantia nigra by combined scanning probe and photoelectron emission microscopies (PEEM). Interpretation of the results is based on previous studies on melanosomes from different tissues and can be summarized as follows:
1) Neuromelanin granules are aggregates composed of substructures of spherical components, 30-nm in diameter, a feature that is shared by most naturally occurring eumelanins, like those from Sepia, human hair and eye melanosomes.
2) Photoelectron emission microscopy, a technique which has proven valuable for distinguishing eumelanin and pheomelanins, indicates a surface threshold ionization potential of ca. 4.5 $\pm$ 0.2 eV, corresponding to an electrochemical oxidation potential of –0.1 $\pm$ 0.2 V vs. normal hydrogen electrode (NHE), which is closer to the oxidation potential of black human hair melanosomes (–0.2 $\pm$ 0.2 V) than to that of hair pheomelanosomes (+0.5 $\pm$ 0.2 V).
On this basis, it is proposed that neuromelanin has a reactive pheomelanin core with reducing properties encapsulated into a eumelanin type spherical architecture.
This paper provides new useful insights into the longlasting issue of neuromelanin structure and function, and the interested reader is also referred to the relevant commentary by S. Ito (Proceedings of the National Academy of Sciences of the United States of America (2006), 103(40), 14647-14648). The results may have a bearing on the complex duality concerning the physiopathological roles of neuromelanin (*viz.* protective vs. cytotoxic, see also Zecca et al., Neurology (2006), 67(7, Suppl. 2), S8-S11). They however raise a number of crucial issues, concerning the origin of this specific architecture, and more generally the ontogeny of neuromelanin granules. Considering that neuromelanin accumulation is a slow process, one could envisage a number of different mechanisms, including a switch from pheomelanin to eumelanin due to a late thiol depletion; alternatively, one could consider that a certain degree of heterogeneity, that is, a certain proportion of eumelanin related components are present also within the core. Another issue concerns the nature of the eumelanin surface component: is this truly indolic in character as in the Sepia, hair and eye melanosomes ? Dopamine is less susceptible to cyclization compared to dopa, and it is possible that in the case of neuromelanin the eumelanin component is slightly different. Consideration of these issues will certainly stimulate further research that will shed new important light on neuromelanin and its role in Parkinson's disease.

- Berg D., Hochstrasser H., Schweitzer K.J., Riess O.
  Disturbance of iron metabolism in Parkinson's disease - ultrasonography as a biomarker. Neurotoxicity Research 9(1):1-13, 2006.
  Abstract :
  A central role of iron in the pathogenesis of Parkinson's disease (PD) has been discussed for many years. So far, however, a biomarker indicating increased iron levels in the substantia nigra (SN) in PD patients has been missing. Performing transcranial ultrasound we detected an increased area of SN echogenicity as a typical echofeature in PD, visible already in the early stages of the disease and in subjects with subclin. impairment of the nigrostriatal system. Animal studies and post mortem analyses of human brain tissue revealed that this echofeature is assocd. with increased iron levels of the substantia nigra as well as a reduced neuromelanin content. The apparently autosomal dominant inheritance of this echofeature in relatives of patients with idiopathic PD indicates a primary role of disturbances of iron metab. in PD. Consequently performed mutation analyses in genes involved in brain iron metab. lead to the discovery of specific mutations in the ferritin-H, IRP2 and HFE gene in single PD patients. Moreover, variations in the ceruloplasmin gene were found to be assocd. with PD or SN hyperechogenicity. Functional relevance of some of these mutations for iron metab. could be proven. Therefore, SN hyperechogenicity can be regarded as biomarker for both: impairment of the nigrostriatal system and increased iron levels of the SN. Future studies aim at substantiating the hypothesis that healthy subjects with SN hyperechogenicity indeed represent a population at risk for nigrostriatal degeneration, which would have a significant impact on therapeutical options.

- Bush W.D., Garguilo J., Zucca F.A., Albertini A., Zecca E., Glenn S., Nemanich R. J., Simon J.D.
  The surface oxidation potential of human neuro melanin reveals a spherical architecture with a pheomelanin core and a eumelanin surface. Proceedings of the National Academy of Sciences of the United States of America.103(40):14785-14789, 2006.
  Abstract:
  Neuromelanin (NM) isolated from the substantia nigra region of the human brain was studied by scanning probe and photoelectron emission microscopies. At. force microscopy reveals that NM granules are comprised of spherical structures with a diam. of 30 nm, similar to that obsd. for Sepia cuttlefish, bovine eye, and human eye and hair melanosomes. Photoelectron microscopy images were collected at specific wavelengths of UV light between 248 and 413 nm, using the spontaneous-emission output from the Duke OK-4 free electron laser. Anal. of the data establishes a threshold photoionization potential for NM of 4.5 0.2 eV, which corresponds to an oxidn. potential of -0.1 0.2 V vs. the normal hydrogen electrode (NHE). The oxidn. potential of NM is within exptl. error of the oxidn. potential

measured for human eumelanosomes (-0.2 0.2 V vs. NHE), despite the presence of a significant fraction of the red pigment, pheomelanin, which is characterized by a higher oxidn. potential (+0.5 0.2 V vs. NHE). Published kinetic studies on the early chem. steps of melanogenesis show that in the case of pigments contg. a mixt. of pheomelanin and eumelanin, of which NM is an example, pheomelanin formation occurs first with eumelanin formation predominantly occurring only after cysteine levels are depleted. Such a kinetic model would predict a structural motif with pheomelanin at the core and eumelanin at the surface, which is consistent with the measured surface oxidn. potential of the 30-nm constituents of NM granules.

- Double K.L., Halliday G.M.
New face of neuromelanin. Journal of Neural Transmission, Supplement 70 (Parkinson's Disease and Related Disorders):119-123, 2006.
Abstract:
The massive, early and relatively circumscribed death of the dopaminergic neurons of the substantia nigra in Parkinson's disease has not yet been adequately explained. The characteristic feature of this brain region is the presence of neuromelanin pigment within the vulnerable neurons. We suggest that neuromelanin in the Parkinson's disease brain differs to that in the normal brain. The interaction of neuromelanin with iron has been shown to differ in the parkinsonian brain in a manner consistent with an increase in oxidative stress. Further, we suggest an interaction between the lipoprotein -synuclein and lipidated neuromelanin contributes to the aggregation of this protein and cell death in Parkinson's disease. The available data suggest that the melaninisation of the dopaminergic neurons of the substantia nigra is a crit. factor to explain the vulnerability of this brain region to early and massive degeneration in Parkinson's disease.

- Gerlach M., Double K. L., Youdim M. B. H., Riederer P.
Potential sources of increased iron in the substantia nigra of parkinsonian patients. Journal of Neural Transmission, Supplement.70 (Parkinson's Disease and Related Disorders):133-142, 2006.
Abstract:
Histopathol., biochem. and in vivo brain imaging techniques, such as magnetic resonance imaging and transcranial sonog., revealed a consistent increase of substantia nigra (SN) iron in Parkinson's disease (PD). Increased iron deposits in the SN may have genetic and non-genetic causes. There are several rare movement disorders assocd. with neurodegeneration, and genetic abnormalities in iron regulation resulting in iron deposition in the brain. Non-genetic causes of increased SN iron may be the result of a disturbed or open blood-brain-barrier, local changes in the normal iron-regulatory systems, intraneuronal transportation of iron from iron-rich area into the SN and release of iron from intracellular iron storage mols. Major iron stores are ferritin and haemosiderin in glial cells as well as neuromelanin in neurons. Age- and disease dependent overload of iron storage proteins may result in iron release upon redn. Consequently, the low mol. wt. chelatable iron complexes may trigger redox reactions leading to damage of biomols. Addnl., upon neurodegeneration there is strong microglial activation which can be another source of high iron concns. in the brain.

- Kim Y.S., Joh T.H.
Microglia, major player in the brain inflammation: their roles in the pathogenesis of Parkinson's disease. Experimental and Molecular Medicine. 38(4):333-347, 2006.
Abstract:
A review with refs. Inflammation, a self-defensive reaction against various pathogenic stimuli, may become harmful self-damaging process. Increasing evidence has linked chronic inflammation to a no. of neurodegenerative disorders including Alzheimer's disease (AD), Parkinson's disease (PD), and multiple sclerosis. In the central nervous system, microglia, the resident innate immune cells play major role in the inflammatory process. Although they form the first line of defense for the neural parenchyma, uncontrolled activation of microglia may directly toxic to neurons by releasing various substances such as inflammatory cytokines (IL-1 , TNF- , IL-6), NO, PGE2, and superoxide. Moreover, our recent study demonstrated that activated microglia phagocytosis not only damaged cell debris but also neighboring intact cells. It further supports their active participation in self-perpetuating neuronal damaging cycles. In the following review, we discuss microglial responses to damaging neurons, known activators released from injured neurons and how microglia cause neuronal degeneration. In the last part, microglial activation and their role in PD are discussed in depth.

- Malagelada C., Ryu E.J., Biswas S.C., Jackson-Lewis V., Greene L.A.
RTP801 is elevated in Parkinson brain substantia nigral neurons and mediates death in cellular models of Parkinson's disease by a mechanism involving mammalian target of rapamycin inactivation. Journal of Neuroscience 26(39):9996-10005, 2006.
Abstract:
The mols. underlying neuron loss in Parkinson's disease (PD) are essentially unknown, and current therapies focus on diminishing symptoms rather than preventing neuron death. We identified RTP801 as a gene whose transcripts were highly induced in a cellular model of PD in which death of neuronal catecholaminergic PC12 cells was triggered by the

PD mimetic 6-OHDA. Here, we find that RTP801 protein is also induced in this and addnl. cellular and animal PD models. To assess the relevance of these observations to PD, we used immunohistochem. to compare RTP801 expression in postmortem brains from PD and control patients. For all PD brains examd., expression was highly elevated within neuromelanin-contg. neurons of the substantia nigra but not in cerebellar neurons. Evaluation of the potential role of RTP801 induction in our cellular model revealed that RTP801 overexpression is sufficient to promote death but does not further elevate death caused by 6-OHDA. Furthermore, RTP801 induction is requisite for death in our cellular PD models and in 6-OHDA-treated cultured sympathetic neurons in that its knockdown by short hairpin RNAs (shRNAs) is protective. The mechanism by which 6-OHDA and RTP801 induce neuron death appears to involve repression of mammalian target of rapamycin (mTOR) kinase activity, and such death is inhibited by shRNAs targeting TSC2 (tuberous sclerosis complex), a protein with which RTP801 interacts to block mTOR activation. Our findings thus suggest that the elevation of RTP801 we detect in PD substantia nigral neurons may mediate their degeneration and death and that RTP801 and its signaling cascade may be novel potential therapeutic targets for the disease.

- Maruyama W., Shamoto-Nagai M., Akao Y., Riederer P., Naoi M.
The effect of neuromelanin on the proteasome activity in human dopaminergic SH-SY5Y cells. Journal of Neural Transmission, Supplement 70 (Parkinson's Disease and Related Disorders):125-132, 2006.
Abstract:
In Parkinson's disease (PD), the selective depletion of dopamine neurons in the substantia nigra, particular those contg. neuromelanin (NM), is the characteristic pathol. feature. The role of NM in the cell death of dopamine neurons has been considered either to be neurotoxic or neuroprotective, but the precise mechanism has never been elucidated. In human brain, NM is synthesized by polymn. of dopamine and relating quinones, to which bind heavy metals including iron. The effects of NM prepd. from human brain were examd. using human dopaminergic SH-SY5Y cells. It was found that NM inhibits 26S proteasome activity through generation of reactive oxygen and nitrogen species from mitochondria. The mitochondrial dysfunction was also induced by oxidative stress mediated by iron released from NM. NM accumulated in dopamine neurons in ageing may det. the selective vulnerability of dopamine neurons in PD.

- Shosuke I., Toyoake A.
Encapsulation of a reactive core in neuromelanin. Proceedings of the National Academy of Sciences of the United States of America. 103(40):14647-14648, 2006.
Abstract:
The research of Bush et al. (2006) entitled "The surface oxidn. potential of human neuromelanin reveals a spherical architecture with a pheomelanin core and a eumelanin surface" is reviewed with commentary and refs. Bush et al. used sophisticated phys. methods, in particular photoelectron emission microscopy (PEEM) coupled to a free-electron laser, to demonstrate that neuromelanin (NM) is composed of granules with diams. of about 0 nm consisting of pheomelanin at the core and eumelanin at the surface. NM is a brown, insol., melanin-like pigment found in the central nervous system of humans and primates. This pigment is present in highest concn. in catecholaminergic neurons of the substantia nigra and locus ceruleus regions of the midbrain. The architecture of NM granules proposed by Bush et al. not only would make interpretation of the existing vast amts. of information on NM possible but also should stimulate renewed interest in the roles of NM in the pathogenesis of Parkinson's disease, the second most prevalent neurodegenerative disorder.

- Zecca L., Zucca F. A., Albertini A., Rizzio E., Fariello R.G.
A proposed dual role of neuromelanin in the pathogenesis of Parkinson's disease. Neurology 67(7, Suppl. 2):S8-S11, 2006.
Abstract:
In many parkinsonian syndromes, neuro melanin (NM)-contg. dopaminergic neurons of the substantia nigra (SN) are selectively targeted by the noxious pathogens. Studies of the constitutional and functional features of human NM allow the formulation of a logical hypothesis on its role in parkinsonian syndromes. In the early stages, NM synthesis and iron-chelating properties may act as a powerful protective mechanism, delaying symptom appearance and/or slowing disease progression. Once these systems have been exhausted, the pathogenic mechanisms affecting cytoplasmic organelles other than NM destroy NM-harboring neurons, with consequent pouring out of NM granules. These in turn activate microglia, causing release of nitric oxide, interleukin-6 and tumor necrosis factor- , thus becoming an important determinant of disease aggravation. Neuro melanin appears to be a suitable target for devising chem. agents that might modify the course of these diseases.

6. Genetics, molecular and developmental biology

(Dr. F. Beermann)

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  EWS-CREB1: a recurrent variant fusion in clear cell sarcoma--association with gastrointestinal location and absence of melanocytic differentiation. Clin Cancer Res 12: 5356-5362, 2006.

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  Characterization of Mahogunin Ring Finger-1 expression in mice. Pigment Cell Res 19: 635-643, 2006.

- Bagheri S, Nosrati M, Li S, Fong S, Torabian S, Rangel J, Moore DH, Federman S, Laposa RR, Baehner FL, Sagebiel RW, Cleaver JE, Haqq C, Debs RJ, Blackburn EH, Kashani-Sabet M.
  Genes and pathways downstream of telomerase in melanoma metastasis. Proc Natl Acad Sci U S A 103: 11306-11311, 2006.

- Bahadori R, Rinner O, Schonthaler HB, Biehlmaier O, Makhankov YV, Rao P, Jagadeeswaran P, Neuhauss SC.
  The Zebrafish fade out mutant: a novel genetic model for Hermansky-Pudlak syndrome. Invest Ophthalmol Vis Sci 47: 4523-4531, 2006.
  Summary: The fad mutant shows syndromic defects in pigmentation, outer retinal structure and function, and blood clotting. This syndrome is characteristic of Hermansky-Pudlak syndrome (HPS), making fad a novel genetic model of HPS. The gene does not cosegregate with the known human HPS genes, suggesting a novel molecular cause of HPS.

- Bharti K, Nguyen MT, Skuntz S, Bertuzzi S, Arnheiter H.
  The other pigment cell: specification and development of the pigmented epithelium of the vertebrate eye. Pigment Cell Res 19: 380-394, 2006.

- Brunberg E, Andersson L, Cothran G, Sandberg K, Mikko S, Lindgren G.
  A missense mutation in PMEL17 is associated with the Silver coat color in the horse. BMC Genet 7: 46, 2006.

- Carney TJ, Dutton KA, Greenhill E, Delfino-Machin M, Dufourcq P, Blader P, Kelsh RN.
  A direct role for Sox10 in specification of neural crest-derived sensory neurons. Development 133: 4619-4630, 2006.
  Summary: Sox10 activity has a role not only for pigment and glial cell lineages, but also for development of neural crest-derived neurons of dorsal root ganglia.

- Chaki M, Sengupta M, Mukhopadhyay A, Subba Rao I, Majumder PP, Das M, Samanta S, Ray K.
  OCA1 in different ethnic groups of india is primarily due to founder mutations in the tyrosinase gene. Ann Hum Genet 70: 623-630, 2006.

- Chin L, Garraway LA, Fisher DE.
  Malignant melanoma: genetics and therapeutics in the genomic era. Genes Dev 20: 2149-2182, 2006.

- Chizhikov V, Steshina E, Roberts R, Ilkin Y, Washburn L, Millen KJ.
  Molecular definition of an allelic series of mutations disrupting the mouse Lmx1a (dreher) gene. Mamm Genome 17: 1025-1032, 2006.
  Commentary: Mice homozygous for the dreher (dr) mutation are characterized by pigmentation and skeletal abnormalities and striking behavioral phenotypes, including ataxia, vestibular deficits, and hyperactivity.

- Cota CD, Bagher P, Pelc P, Smith CO, Bodner CR, Gunn TM.
  Mice with mutations in Mahogunin ring finger-1 (Mgrn1) exhibit abnormal patterning of the left-right axis. Dev Dyn 235: 3438-3447, 2006.

- Denoyelle C, Abou-Rjaily G, Bezrookove V, Verhaegen M, Johnson TM, Fullen DR, Pointer JN, Gruber SB, Su LD, Nikiforov MA, Kaufman RJ, Bastian BC, Soengas MS.
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  In Melanoma, RAS Mutations Are Accompanied by Switching Signaling from BRAF to CRAF and Disrupted Cyclic AMP Signaling. Cancer Res 66: 9483-9491, 2006.
  Abstract: Melanocytes require the RAS/RAF/MEK/ERK and the cyclic AMP (cAMP) signaling pathways to maintain the fine balance between proliferation and differentiation. We have investigated how cross-talk between these pathways

affects melanoma progression. We show that cAMP suppresses CRAF activity in melanocytes and that this is essential to suppress the oncogenic potential of CRAF in these cells. As a consequence, BRAF alone is responsible for signaling to MEK. However, when RAS is mutated in melanoma, the cells switch their signaling from BRAF to CRAF. This switch is accompanied by dysregulated cAMP signaling, a step that is necessary to allow CRAF to signal to MEK. Thus, a fundamental switch in RAF isoform usage occurs when RAS is mutated in melanoma, and this occurs in the context of disrupted cAMP signaling. These data have important implications for the development of therapeutic strategies to treat this life-threatening disease.

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FVB.129P2-Pde6b(+) Tyr(c-ch)/Ant, a sighted variant of the FVB/N mouse strain suitable for behavioral analysis. Genes Brain Behav, online 2006.

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Shroom2 (APXL) regulates melanosome biogenesis and localization in the retinal pigment epithelium. Development 133: 4109-4118, 2006.

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Mutations in the melanocortin 1 receptor (MC1R) gene are associated with coat colours in the domestic rabbit (Oryctolagus cuniculus). Anim Genet 37: 489-493, 2006.

- Girardot M, Guibert S, Laforet MP, Gallard Y, Larroque H, Oulmouden A.
The insertion of a full-length Bos taurus LINE element is responsible for a transcriptional deregulation of the Normande Agouti gene. Pigment Cell Res 19: 346-355, 2006.

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lineage-directed gene transfer, we show that, in the mouse, Mitf can rescue Sox10-mutant precursor cells only partially. In fact, retrovirally mediated, Sox10-independent Mitf expression in mouse melanoblasts leads to cell survival and expression of a number of pigment biosynthetic genes but does not lead to expression of tyrosinase, the rate-limiting pigment gene which critically depends on both Sox10 and Mitf. Hence, compared with fish, mice have evolved a regulation of tyrosinase expression that includes feed-forward loops between Sox10 and tyrosinase regulatory regions. The results may help to explain how some embryos, such as zebrafish, can achieve rapid pigmentation after fertilization, whereas others, such as mice, become pigmented only several days after birth.

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7. Tyrosinase, TRPs, other enzymes

(Prof. J.C. Garcia-Borron)

8. Melanosomes

(Prof. J. Borovansky)

Reviews of various length covering pigmentation genes (*Sturm*), melanocytes (*Goding*), mouse coat colour mutations (*Steingrímson et al*), hypopigmenting agents (*Solano et al*) and properties of melanin (*Sarna&Meredith*) have, of course, mentioned melanosomes in various aspects. The extent of our knowledge of melanosomal constituents has always reflected the degree of homogeneity and nativity of isolated organelles: A qualitative increase of melanosome proteomics has been brought by *Chi et al*. A fundamental catalogue of primary importance of lysosome-related organelles (including melanosomes) proteomes was put together (*Hu et al*). Shroom 2 (APXL) participation in melanosome localization in the RPE was demonstrated by *Fairbank et al*. Melanosomes defects as well as defects of vesicular trafficking were observed in neurocutaneous syndrome by *Buoni et al*. *Hirobe&Abe* brought further support for our hypothesis that not the pigment but the quality of organelle matrix is decisive for melanosome shape and ultrastructural appearance. *Hong et al* announced damage and/or degradation of aged RPE melanosomes and a deposition of lipofuscin on the surface of RPE melanosomes. Photodegradation and subsequent chemical changes of RPE melanosomes were analyzed by *Zareba et al.* A never-ending story of special role(s) of zinc ions in the melanosome and melanocyte and their cytotoxicity has continued by the observation of *Plonka et al.*. Melanosomes can act as energy-converting device: *Pustovalov & Jean* in model computer experiments derived equations for calculations of time dependence of spheroid particle temperature and laser pulses.

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  Comments: Melanosome biogenesis was studied in neonatal epidermal melanocytes (Dct+/Dct+) and in congenic mutant melanocytes (Dct(slt)/Dct(slt) grown in serum-free primary cultures. . EM revealed that wild type melanocytes possessed exclusively elliptical melanosomes with longitudinal internal structures (i.e. lamellar melanosomes), whereas in mutant melanocytes numerous spherical melanosomes with globular melanin depositon (i.e. granular melanosomes) but also elliptical and mixed-type melanosomes were present. Mutant melanocytes contained less Stage IV melanosomes.

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  Comments: A review describing pigmentation genes and human population allele frequencies. It contains figures illustrating melanosome formation and the role of ion transport in their maturation as well as the distribution of melanosomes in keratinocytes of different ethnic origin. A special attention was paid to zebrafish *golden* mutation characterized by the production of small and irregular-shaped melanin granules.

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  Comments: Isolated porcine RPE melanosomes subjected to a high intensity visible light were shown to suffer from morphologic changes consistent with photodegradation – reduction in their electron density, particle fragmentation and surface disruption. Illuminated melanosomes had lower melanin content and reduced ability to bind iron but increased ability to photogenerate superoxide anions. (As for melanosome and melanin photodegradation see also *Elleder M- Histochem J 33(5):273-281, 2001*).

ANNOUNCEMENTS & RELATED ACTIVITIES

Calendar of events

2007 68th Annual Meeting for the Society for Investigative Dermatology

May 9-12, Los Angeles, California

Contact: Carolyn Slade
Meetings Coordinator
Tel: 216-589-0917 Fax: 216-579-9333
E-mail: slade@sidnet.org

2007 16th Congress of the European Academy of Dermatology and Venereology

May 16-20, Vienna, Austria

Contact: Prof. Erwin Tschachler
Congress President
16th EADV Congress
E-mail: president@eadvvienna2007.com

2007 2nd Conference of the Asian Society for Pigment Cell Research (ASPCR)

Incorporating :

20th Annual Scientific Meeting of the Dermatological Society of Singapore

July 6-8, Singapore

Contact: Conference Secretariat, Mrs Alice Chew
National Skin Centre
1 Mandalay Road
Singapore 308205
Tel: (65) 6350 8405; Fax: (65) 6253 3225
E-mail: training@nsc.gov.sg
Web: http://www.aspcr.org/ASPCR2007

2007 37th Annual ESDR Meeting

September 6-8, Zurich, Switzerland

Contact: E-mail: office@esdr.org
Web: www.esdr.ch

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

September 13-16, Chicago, IL, USA

Contact: Caroline LePoole
E-mail: ilepool@lumc.edu
Web : paspcr.med.umn.edu/

2007 21st World Congress of Dermatology

October 1-5, Buenos Aires, Argentina

Contact: E-mail: info@dermato2007.org
Web: www.dermato2007.org

2007 XIVth Meeting of the European Society for Pigment Cell Research

October 14-17, Bari, Italy "Pigment Cells and their environment"

Contact: Prof Rosa Cicero
E-mail : r.cicero@biolgene.uniba.it
E-mail : espcr@gruppotriumph.it
Web: www.espcr.org

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

December 8-9, Toyoake City, Japan

Contact: Prof. Kazumasa Wakamatsu
Web: wwwsoc.nii.ac.jp/jspcr/

2008 20th International Pigment Cell Conference (IPCC)

conjoined with

IMR2008 Vth International Melanoma Research Congress

May 7–12 Sapporo, Japan

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

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

May 14-17 , Kyoto, Japan

Contact: E-mail: office@esdr.org
Web: www.esdr.ch

2009 Annual Meeting for the Society for Investigative Dermatology

May 6-9, Montreal, Quebec, Canada

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NEW MEMBERS

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

COSGAREA Rodica
Dept of Dermatology
University of medicine and pharmacy
Cluj-Napoea
Clinicilor 3-5
RO - 400006 CLUY-NAPOCA
tel: 40-744700559
Fax: 40-264592394
e-mail: mcosgarea@cluj.astral.ro

CREPALDI Paola
Institut Zootechnia generale
Facolta agraria, Universita Milano
Via Celoria 2
I - 20133 MILANO
Tel: (39)0250316442
Fax: (39)0250316434
e-mail: paola.crepaldi@unimi.it

PUIG Isabel
Institut Curie
UMR 146 du CNRS
Bat 110, Centre Universitaire
F - 91405 ORSAY cedex

RICHARDS Jeanette
Beaty Technology Division
The Procter and Gamble Company
11810 East Miami River Road
USA - 45252 CINCINNATI, OHIO
Tel: (513)627-0513
Fax: (513)627-0238
e-mail: richards.ja.1@pg.com

SCHNAEKER Eva-Maria
University of Muenster
Dept of Dermatology
Von Esmarch strasse 58
D - 48149 MÜNSTER
Tel: 0251-8352685
Fax: 0251-8357279
e-mail: ems@uni-muenster.de

SCHOUWEY Karine
ISREC
Ch. des Boveresses 155
CH - 1066 EPALINGES
Tel: (+41)216925831
Fax: (+41)216526933
e-mail: karine.schouwey@isrec.ch