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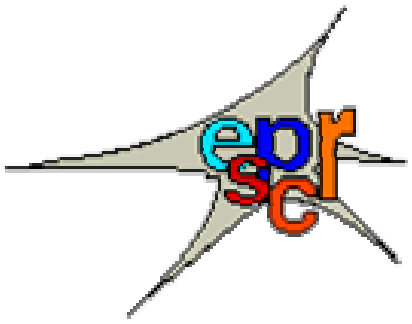

EUROPEAN SOCIETY FOR PIGMENT CELL RESEARCH *BULLETIN*

N° 54 - April 2006

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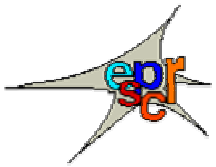


LETTER TO THE EDITOR
DISCUSSION, REVIEW,
SHORT COMMUNICATION, ...

MEETING REPORT
19th IPCC - Sep 18-22, 2005
Reston, Virginia

Sunday, September 18, 2005

Plenary Symposium 1 - "Advancing the Frontiers"

by John Pawelek

Keynote Lecture 1: "Genomics and Disorders of Human Pigmentation", Dr. FS Collins, National Human Genome Research Institute

Dr. Francis Collins summarized some of the major achievements of the Human Genome Project with the completion of its original goals in 2003. Some 30 mammalian genomes have now been sequenced. One of the most striking findings is the well-known remarkable similarity (98.9%) of the DNA sequences between humans and chimpanzees. There are now many services and gene libraries available to scientists. Currently in the public repository are more than 22, 000 human loci and 23, 000 mouse loci. The Knock-Out Mouse Project has the goal of establishing knock-outs for all mouse genes. The ENCODE project is an encyclopedia of human DNA elements. The Molecular Libraries Roadmap has screening centers that can screen 100,000 small molecules for activity in enzymatic assays of your design. These molecules can be accessed in the new PubChem database. The Human Cancer Genome Project will sequence the genomes of 250 representative tumors from each of 50 tissue types. The International HapMap Project (www.hapmap.org) is designed to better characterize the single nucleotide polymorphisms (SNP's) in the human genome.

Keynote Lecture 2: Telomeres, Telomerase, Senescence and Cancer", Dr. EH Blackburn, University of California, San Francisco

Dr. Blackburn summarized the role of telomerase in maintaining telomere length and lifespan extension in human cancer cells. One surprising finding was that net telomere lengthening and lifespan extension can be uncoupled, supporting the conclusion that telomerase plays roles in addition to the net lengthening of telomeric DNA. This came from an unexpected finding that a hairpin siRNA targeting human telomerase RNA rapidly inhibited growth of cancer cells without bulk telomere shortening and induced a novel gene expression that included suppression of genes implicated in angiogenesis and metastasis. The findings uncovered functions of telomerase in tumor growth and progression in addition to telomere maintenance.

Keynote Lecture 3: "Frontiers in fluorescent Protein Imaging of Living Cells", Dr. J. Lippincott-Schwartz, National Institute of child Health and Human Development.

Dr. Lippincott-Schwartz detailed the use of fluorescent proteins such as green fluorescent protein (GFP) as molecular tags for following complex biological processes in living cells. Modified GFP's have been used as markers to track and quantify individual or multiple protein species, as probes to monitor protein-protein interactions, and as photochemically-modulatable proteins to highlight and follow the fate of specific protein populations within the cell. She focused on methods of kinetic microscopy involving photobleaching and photoactivation that are being used to monitor the appearance, location, movement, and degradation of GFP fusion proteins.

Plenary Symposium 2 – "Hot Topics Symposium"

by Shosuke Ito, Stan Pavel, and Vincent J. Hearing

Plenary Symposium 2 was co-chaired by the Organizers of the current and the two previous IPCCs: Shosuke Ito, Stan Pavel, and Vincent J. Hearing. The tradition of holding the "Hot Topics Symposium" was introduced during the 17th IPCC held in Nagoya six years ago. The idea was to put together the best abstracts submitted with highly innovative contents that would be selected from all abstracts accepted for oral presentation in the various Symposia. It was an enjoyable yet (tough)

uneasy job to anonymously choose the 5 best abstracts from the 15 already selected as #1 for each of the 15 symposia. Stan, Vince, and I were pleased to select the following 5 abstracts that will certainly advance further our knowledge on pigmentation and pigment cells. We take special delight in noting that the 5 abstracts chosen were from 5 widely divergent areas and that there was an excellent distribution of speakers internationally which shows the high quality of science being performed around the world.

The first presentation was given by Dr. Victor A. Canfield (Hershey, USA) who talked about the identification and characterization of zebrafish *golden* gene. This newly identified gene encodes a potassium-dependent sodium, calcium exchanger. Surprisingly, the human orthologue is involved in the regulation of constitutive pigmentation. It was shown that 30% variation in human pigmentation may be attributable to one of the alleles of the *golden* gene.
In the next presentation, Dr. Emi Nishimura (Hokkaido, Japan) discussed the mechanisms of hair graying which is everybody's concern. She was able to demonstrate that hair graying is caused by defective self-maintenance of melanocyte stem/progenitor cells. In *Bcl*-deficient mice, hair turns gray with aging because melanocyte stem cells selectively die by apoptosis in the stem cell niche in dormant state. These processes are controlled by the melanocyte master transcription regulator *Mitf*.

In the following talk, Dr. Mitsunori Fukuda (Wako, Japan) discussed the roles of Slp- and Slac2-family proteins in melanosome distribution and maintenance of elongated shape of melanocytes. Among others, knockdown of endogenous Slp-2a, the most abundant of the Slp family, by siRNA caused a dramatically reduced number of melanosomes in the cell periphery of melanocytes ("peripheral dilution") and a morphological change to a round shape. These processes mimicked those seen in Griscelli syndrome.

A happening took place at the beginning of the next talk by Dr. Guillaume Robert (Nice, France). A fire alarm went off, and everybody was forced to rush out of the building. After having watched fire engines arriving and firemen working, there was nothing to worry about. We returned to the room and resumed the symposium with a full audience. Dr. Robert discussed the roles of SPARC, Secreted Protein Acidic and Rich in Cysteine. SPARC expression is inversely correlated to E-cadherin expression in melanocytes and malignant melanoma cell lines. SPARC depletion leads to up-regulation of E-cadherin, and SPARC-null cells exhibit a marked decrease in their migratory and invasive phenotype, supporting a critical role for SPARC in malignant transformation of normal melanocytes.

Finally, Dr. James Grichnik (Durham, USA) closed the Symposium by addressing the most controversial topic among the five talks. He presented a hypothesis that melanomas are derived from stem cells and not through stepwise "dedifferentiation" from melanocytes. Melanoma cell lines investigated revealed the presence of a subpopulation of small cells that were less pigmented and grew slowly to form colonies. These and other findings support the possibility of existence of melanoma tumor stem cells and have implications for the origin of melanoma.
Everybody found that the Hot Topics Symposium was successful as it was in Nagoya and Egmond aan Zee, and were ready for the Welcome reception with the 20 min's delay.

Monday, September 19, 2005

Plenary Symposium 3 - "Developmental Biology 1, Melanoblast/RPE - Specification, Development, Survival and Apoptosis (Waardenburg syndrome, Tietz syndrome)"

by Veronique. Delmas

The symposium "Developmental Biology of Melanocytes" opened with two consecutive plenary lectures. The first was presented by E. Dupin who described the plasticity exhibited by the Neural

Crest Cells (NCC) *in vivo*, and the presence of precursor cells in Neural Crest derivatives until late in development. Quail neural crest cells isolated at early migratory stages are heterogeneous with respect to their developmental potential, including lineage-committed cells as well as diverse pluripotent and oligopotent progenitors. The reversibility of differentiated cells (melanocytes or glia) to their pluripotent precursor can be induced *in vitro* by endothelin 3. Therefore, when subjected to appropriate stimulus, pigment cells can revert to their neural crest stem cell ancestors.

The second plenary lecture was delivered by H. Arnheiter who summarized a large body of work from his own lab and others on the essential role of MITF in pigment cell development. H. Arnheiter reported a novel and interesting way of regulating MITF activity by its association with the histone deacetylase HDAC proteins. The specific role of HDAC members in melanocyte development *in vivo* is under further investigation in his laboratory.

Next J. Lister addressed the function of the forkhead transcription factor, Foxd3 during zebrafish NCC fate specification and suggested that in culture Foxd3 can repress *Mitfa* promoter in a melanoma cell line.

From the same lab, K. Bismuth described the generation of a knock-in mouse whose *Mitf* gene encodes a non-phosphorylatable alanine instead of serine (S73). Surprisingly, the introduced mutation leads to preferential exclusion from the mRNA of the subexon 2b which encodes the mutated S73A residue. The predominant expression of MITF lacking exon 2b, along with a minor contribution of full-length MITF lacking a phosphorylatable S73, increases the numbers of differentiated melanocytes.

Several presentations described the function and the relationship/interconnection between the genes involved in the Waardenburg syndrome type 4, an auditory-pigmentary disorder which is characterized with the presence of the aganglionic megacolon. These genes are the *endothelin receptor type B* (*EDNRB*) gene, the *endothelin 3 (EDN3)* gene, or the *SOX10* gene. S. Yokoyama described how Sox10 regulates the endothelin receptor type B in pigment cells. He reported that SOX10 can transactivate two of the four *EDNRB* promoters by different mechanisms; an Sp1-dependent mechanism for the conventional *EDNRB* promoter and by an Sp1-independent mechanism for the *EDNRBΔ2* promoter. MK Lowenstein (L. Kos's laboratory) described the generation of a new transgenic mouse line expressing the *Endrb* under the control of the *Dct* promoter which is able to rescue the hypopigmentation phenotype of the heterozygous *Sox10* mutant, $Sox10^{tm1Weg}/+$, but not the phenotype of Pax3 heterozygous mutants. These results suggest that Ednrb and Sox10 might interact specifically for proper melanocyte development.

Plenary Symposium 3 - "Developmental Biology 1, Melanoblast/RPE - Specification, Development, Survival and Apoptosis" (Waardenburg syndrome, Tietz syndrome)

by Lidia Kos

In her plenary lecture, E. Dupin reviewed a series of experiments that demonstrate the initial heterogeneity of neural crest precursors. Avian neural crest precursors range from totipotent to lineage-committed cells, all with the potential to give rise to melanocytes. Some of these precursor cells exhibit stem cell properties and are dependent on the action of Endothelin 3 for their survival and proliferation. In vitro, already committed melanocytes maintain the capacity to respond to this factor by up-regulating a series of early neural crest markers and returning to a bipotential (melanocyte-glia) or pluripotent state. Dr. Dupin suggested that this plasticity might have some in vivo significance in cases such as nerve injury when melanocytes and glial cells might need to re-program to compensate for cell loss.

Very little is known about the regulation of the Endothelin 3 receptor, endothelin receptor b (EDNRB). S. Yokoyama et al showed that in human melanocyte cell lines, the transcription factor Sox10 was

capable of transactivating the conventional EDNRB as well as the alternative EDNRBΔ2 promoters. Sox10 could bind to two CA-rich regions in the conventional EDNRB promoter and its transactivation was synergistically enhanced by the binding of SP1 to a GC box. In further support of a possible interaction between Sox10 and Ednrb, M. K. Lowenstein et al. showed that the transgenic expression of Ednrb under the control of the DOPAchrome tautomerase promoter (Dct-Ednrb) was capable of rescuing the hypopigmentation phenotype of heterozygous mutant mice in which LacZ was inserted into the Sox10 locus ($Sox10^{tm/Weg}$). The rescue happened as early as embryonic day 12.5 as more melanoblasts were observed in the trunk region of Sox10 mutant embryos carrying the Dct-Ednrb transgene when compared to those without the transgene. However, heterozygous mutant mice for both Sox10 and Ednrb did not exhibit an increase in their hypopigmentation phenotype when compared to either mutant alone, suggesting that at the genetic level these two genes do not act synergistically for the production of a normal coat color pattern.

The transcription factor MITF plays a critical role in all stages of melanocyte development, controlling cell determination, survival, proliferation and differentiation. In his plenary lecture, H. Arnheiter provided a series of genetic evidence in mice to highlight the importance of the tight regulation that MITF undergoes both at the transcriptional and post-translational levels that allows this factor to exert so many different functions. He focused specifically on the mechanism of gene regulation provided by the control of the acetylation state of nucleosomal histones mediated by histone acetyltransferases and histone deacetylases (HDACs). HDACs are expressed in various melanocyte cell lines and MITF overlaps with HDACs 1 and 4. A small domain in HDAC 4 can bind to MITF in vitro and this interaction resulted in the repression of MITF directed transcription of target genes. Dr. Arnheiter suggested that the pigmentation phenotypes of the many MITF mutant alleles could possibly be explained by their interactions with HDACs.

The other two talks further addressed mechanisms of MITF regulation. In vitro studies have shown that Kit signaling regulates MITF by phosphorylation at Serine 73 (S73), increasing both its transcriptional activity and its degradation. K. Bismuth et al. created a knock-in mouse in which S73 was substituted by a non-phosphorylatable alanine. The mutated codon leads to the exclusion of exon 2b from the mRNA, indicating that this region is part of an exonic splicing enhancer. Homozygous mutant mice displayed normal coat color pattern but neural crest cell cultures derived from mutant embryos gave rise to many more melanocytes when compared to wild type cultures, suggesting that MITF lacking exon 2b increases the proliferation and/or survival of precursors. J.A. Lister et al. presented evidence that in zebrafish, mitfa is negatively regulated by the transcription factor foxd3, an early neural crest marker. The zebrafish mitfa mutant nacre lacks melanophores but shows an increase in the number of iridophores. When foxd3 was knocked down by morpholinos, the number of iridophores was reduced in wild type animals but not in mitfa mutants, suggesting that in this pigment cell type foxd3's effect is mediated via the repression of mitfa. This putative interaction seems to be direct as demonstrated by the ability of foxd3 to repress the activity of the mitfa promoter in vitro.

Plenary Symposium 4 - "Evolution and Development of the Pigmentary System"

by Randall L. Morrison

Greg Barsh gave the *Aaron B. Lerner lecture* at the beginning of this session and discussed the coat color genetics of dogs. Agouti pigment type switching does occur in dogs and mahogany and mahganoid are involved as well. The dsk genes associated with dermal pigmentation also play a role as well and many of these genes seem to be acting through a Gq-mediated phospholipase C pathway. When double mutants between Dsk1 and MC1R were examined it was discovered that they promoted pigment type switching in the same direction. Dogs have a black coat that was thought to be caused by a dominant Agouti allele, but this was discovered not to be the case. Black coat color in German Sheperds was found to be caused by a recessive Agouti allele as is seen in other mammals. A genome scan of a Labrador x Greyhound cross identified a new locus responsible for pigmentation in dogs that

they have called the “K” locus. Variability of the K locus is responsible for the brindled and fawn color patterns seen in some dogs. K is also epistatic to Agouti which suggests this is novel interacting pathway regulating dog coat color.

Shin-Ichi Nishikawa gave the second keynote lecture in this session on the regulation of melanocyte stem cells by the stem cell niche. It is not well understood how the stem cell niche maintains the quiescence of melanocyte stem cells (MSCs). A Dct promoter was used to drive GFP expression which then acted as a marker for cell sorting of melanocytes. This allowed the isolation of specific populations of melanocytes. Differentiated cells were then depleted using an anti-c-kit antibody, leaving just the MSCs. These cells exhibit a down-regulation of a number of melanocyte specific markers. The bulge of the hair follicle is high in Wnt inhibitors produced by the melanocyte stem cells. Notch signaling is also active in MSCs. DAPT, a γ-secretase inhibitor, induces melanoblast cell death, but Hes1 over-expression acting through the Notch pathway can rescue cells treated with DAPT. Forced over-expression of Hes1 also causes hair graying. These data suggest that Notch signaling is important for MSC maintenance in the stem cell niche.

Shigeki Shibahara gave the *Makoto Seiji lecture* to end the session and talked about stress responses in mice involving Mitf. The black-eyed white (bw) mouse is an Mitf mutant with a white coat and inner ear defects. The mutation is an insertion of a L1 element into the third intron of the gene. These mice exhibit some unexpected differences. Whole body plethysmyography demonstrated a changed ventilatory response, they have a low respiratory frequency and large tidal volume. There is apparently no altered activity of the neural crest derived oxygen sensing cells, but there is altered chemosensitivity in the brain. Lipocalin-type prostaglandin D2 synthase (L-PDGS) mRNA is not expressed in bw mice. Mitf siRNA decreases L-PDGS expression in B16 melanoma cells. L-PDGS is apparently regulated by Mitf by a cluster of E boxes. L-PDGS is known to be involved in sleep and pain perception. There are indeed behavioral differences in bw mice that include increased activity in the morning. The talk ended with the speculation that via chemosensing and regulation of L-PDGS that perhaps Mitf regulates murine quality of life!

Plenary Symposium 5 - "Developmental Biology 2"

by F.Beermann

The developmental biology session 2 consisted of 2 plenary lectures and 4 oral presentations.
Lionel Larue (Orsay, France) reported on the analysis of ß-catenin and melanocyte development. When a dominant-active ß-catenin was expressed in transgenic mice under control of tyrosinase regulatory elements, the resulting mice unexpectedly showed reduced pigmentation and a white belly spot. Apparently, nuclear ß-catenin thus inhibited melanoblast differentiation. Moreover, ß-catenin expressing cells immortalized efficiently due to silencing of the $p16^{INK4a}$ promoter, which contains binding sites for the Lef transcription factor. In consequence, the presence of the activated nuclear ß-catenin was sufficient to induce melanomagenesis in combination to an activated N-ras transgene.
Bill Pavan (Bethesda, MD, USA) introduced a mutagenesis screen in mice looking for interacting partners of the transcription factor Sox10. In the screen, he took advantage of a knock-in allele of Sox10 which leads to Sox10-deficiency but retaining Sox10-specific lacZ expression. This allowed to analyse 1. dominant enhancers of Sox10-mutant coat color phenotype, 2. changes in Sox10 embryonic expression pattern (as seen by lacZ staining) and 3. Sox10-mediated lethality. Several loci were identified and mapped to specific mouse chromosomes. Some mapped to known genes, as for example Gli3, which was not yet implicated as a coat color gene, whereas other mutations will most probably be new loci.
T.Kunisada (Gifu, Japan) reported on a mouse model for hair graying, the $Mitf^{vit/vit}$ mouse, which is characterized molecularly by a mutation in the DNA-binding domain of Mitf. Using transgenic expression of hepatocyte growth factor or stem cell factor, but not endothelin-3, the hair graying

phenotype and the whitening of the mice could be suppressed suggesting that they might contribute to the self renewal of melanocyte stem cells in the niche.
S. Mirabal (Miami, FL, USA) generated Ednrb transgenic mice under control of the nestin regulatory sequences. These mice show a hypopigmentation phenotype including a belly spot. This phenotype could be rescued by inducing expression of the ligand for Ednrb, Edn3, in keratinocytes.
D.L.Silver (Bethesday, MD, USA) reported on molecular analysis of the belted mutation, which is caused by mutations in the ADAMTS20 gene (" a secreted disintegrin-like and and metalloproteinase"). The belted mutations appears to act synergistically with Pax3 and c-kit mutations, thus possibly modifying kit-receptor/kit-ligand interaction. Moreover, Adamts20 might affect melanoblast development by affecting processing/ expression of a chondroitin sulfate proteoglycan called versican.
I. Fernandez (Miami, FL, USA) studied the role of erbB3 receptors in melanocyte development, using mainly neural crest cultures. Using the ligand neuregulin in cultures, the number of Tyrp1- and Dct-positive cells increased suggesting that neuregulin, via erbB3 might act as a proliferation factor for melanoblasts in vivo.

Concurrent Session 1 – "Genetics of Pigmentation"

Chaired by: Ian Jackson, Murray Brilliant and Richard Sturm

Genetic studies of pigmentation have greatly benefited over the years by studies of model organisms. Indeed, the early work on genetics in a range of organisms has often been the study of pigmentary variation, and there is a rich source of information to be found among these variants. The speakers in this concurrent session spoke about genes in mammals ranging from mice through cats and dogs to cattle and humans.

Teresa Gunn (Cornell) previously showed that gene affected in mahogany mutant mice was the extracellular protein, attractin. At this meeting she described an attractin-related gene in the mouse genome, in which mutations produce the same dark coat and neurodegeneration phenotypes as mahogany. Further along the "predator pathway", Anna Schmidt-Kuntzel (NCI Frederick) described the identification of mutations in the Tyrp1 gene of cats. The chocolate mutation has two molecular defects; a mutation 5 bases inside an intron which results in the variable addition of 51 or 54 bases to the mRNA (and presumably an protein insertion of 17 or 18 amino acids) but also an Ala to Gly substitution in the signal sequence. It appears that even these two changes do not cause complete loss of function; there is a more severe colour phenotype, cinnamon, that is due to a nonsense mutation at codon 100. Maintaining the predator metaphor, Sheila Schmutz (University of Saskatchewan) discussed some coat colour variants of dogs, in particular the dilution phenotype seen in the Shar-Pei and Large Munsterlander breeds. The dilution appears to be due to mutation of the melanophilin gene, which underlies the leaden mouse mutation and some forms of Gricelli Syndrome in humans. The mutant dog gene contains only a synonymous change in the coding region, close to an intron, but RT-PCR reveals aberrant splicing. Also from University of Saskatchewan, Kim MacLean described cattle breeding data that suggest an association between MC1R genotype and traits desirable in meat production.

Moving on to human disease, Marjan Huizing (NIH) described her work screening Hermansky-Pudlak patients. There are, to date, seven genes that have been identified as mutated in HPS patients, and by screening the exons of these she identified mutations in 90% of a collection of 150 patients. Interestingly, mutations in these remaining 15 individuals could not be found, even after screening the human homologues of the genes affected in the additional nine or so mouse HPS models. Perhaps these remaining patients will provide mutation data on control elements of the genes. Tamio Suzuki (Nagoya University) described HPS patients in Japan. Mutation of the HPS1 gene appears to be the commonest form of the disease, and he has found a number of novel mutations. One previously-described splice site mutation was common in the population, most likely the result of a founder effect.

Two speakers addressed the genes responsible for normal variation in human pigmentation. Carolina Bonilla (Ohio State University) extolled the power of admixed populations for genetic association studies. A study using a series of ancestry informative markers on several admixed populations revealed a correlation between skin pigmentation and the contribution of African ancestry (indicating the multigenic nature of pigmentation). Several genes could be identified that showed significant contributions; including the known pigmentation genes, ASIP, TYR, OCA2 and MATP. Justin Graf (Queensland University of Technology) described in more detail polymorphisms of one of these, MATP. Two coding polymorphisms have been identified in the Caucasian population, and homozygosity for the rarer alleles of both show an association with dark hair and olive skin (although as these are the rarer forms the number of homozygotes in the population is small). However, he described a much commoner polymorphism upstream of the coding region that is associated with skin colour variation, independent of hair and eye colour.

The session demonstrated once again the power of genetics to discover gene function, and the diversity of model organisms available to researchers.

Concurrent Session 2 – "Biochemistry of Melanogenesis"

By Bryan B. Fuller

Functional HPA Axis Homolog is Expressed by Melanocytes. A Slominski, B. Zbytek, M. Zmijewski, and J. Wortsman,

This paper, presented by Dr. Slominski, presented data on the expression in the skin of hormones and receptors typically found in the hypothalamo-pituitary-adrenal (HPA) axis. Results showed that human keratinocytes expressed corticotrophin releasing hormone (CRH) as well as the CRH-R1 receptor. The authors also found, as had been shown previously, that keratinocytes produce POMC (Pro-opiomelanocortin). Although one could envision an endocrine loop that involves the CRH induced production of POMC in keratinocytes, the authors found that CRH does NOT up regulate POMC in these cells. Further, no corticosteroids are produced in keratinocytes by CRH. Further studies showed that CRH could increase cAMP levels in human melanocytes and dermal fibroblasts and cause an increased production of POMC and ACTH. The importance of the CRH-R1 receptor in mediating this effect of CRH was demonstrated by blocking the CRH-R1 receptor with the specific antagonist, antialarmine, and showing that no POMC or ACTH was produced. Both melanocytes and fibroblasts were found to respond to either CRH or ACTH with enhanced production of corticosteroids although in fibroblasts, ACTH is the primary stimulator of corticosterone production.

A Novel 43 kDa Protein as a Negative Regulatory Component of Phenoloxidase-Induced Melanin Synthesis. M. Zhao, I. Soderhall, J.W. Park, Y.G. Ma, T. Osaki, C.H. Ha, C.F. Wu, K. Soderhall and B.L. Lee.

Phenoloxidase is widely distributed among animals, plants, and fungi, and is involved in many biologically essential functions. In arthropods, melanization plays an important role in defense reactions, such as wound healing, encapsulation, sequestration of microbes, and the production of toxic intermediates, that are speculated to kill invading microorganisms. In response to injury a melanization reaction occurs at the site of injury and the area wounded by invading microorganisms becomes blackened because of the de novo synthesis and deposition of melanin. In this presentation, the authors show that the activity of phenoloxidase and the production of melanin may be under negative regulatory control by a newly discovered protein (43kDa). The authors report the cDNA cloning of this protein from the mealworm and show that it has no homology to any known sequence. The protein is referred to as MIP (melanization inhibiting protein) and the authors have shown that a recombinant form of this protein can inhibit melanin synthesis in vitro. Further, if a double-stranded inhibitory

RNA is injected in mealworm larvae to block the production of MIP, melanin synthesis increases. The mechanism of action of the 43kDa protein is not known.

Down-Regulated Melanogenic Paracrine Cytokine Linkages in Hypopigmented Palmoplantar Skin". J. Hasegawa, Y. Goto, H. Murata, M. Takata, T. Saida, and G. Imokawa.

Recent studies from Dr. Hearing's laboratory have shown that at least one reason for the low melanocyte density in palmoplantar human skin (five times lower than that found in non-palmoplantar sites) may be that palmoplantar fibroblasts express high levels of dickkopf-1 (DKK1). This protein may then decrease melanocyte proliferation and pigmentation by inhibiting the Wnt signaling pathway. Further studies by Hearing's group showed that transfection of DKK1 decreased melanocyte function, apparently through a ß-catenin–mediated regulation of MITF. In the paper by Dr. Hasegawa, results are presented that show a decreased expression of melanogenic factors by keratinocytes and fibroblasts in palmoplantar skin. Immunohistochemical analysis revealed not only a decrease in the numbers of tyrosinase positive cells in palmoplantar skin, as would be expected, but also a decreased expression of SCF, ET-1, c-KIT and ET-R in palmoplantar skin. Interestingly, the authors present data that MITF levels are similar in palmoplantar and non-palmoplantar skin. Further, in contrast to published findings on DKK-1 levels in palmoplantar fibroblasts, the results presented by Dr. Hasegawa, show that the DKK-1 protein is not detectable in any palmoplantar or non-palmoplantar skin. This finding raises the question of the inhibitory role of DKK-1 in preventing proliferation and differentiation of melanocytes in palmoplantar skin.

Concurrent Session 3 - "Intracellular Signaling"

by Hee-Young Park

Mizutani et al. from Tokyo Women's Medical College presented results that p38 activation is mainly responsible for UVB-induced increase in c-kit expression in human melanocytes. When human melanocytes were UVB (40-80 mJ/cm^2) irradiated, the c-kit expression was increased at both gene and protein level at 12-24 hours after the irradiation. AP-2α, the transcription factor c-kit, was concomitantly increased. Inhibitors of PKA (H-89), PKC (Calphostine), MAPK (PD98059), JNK (SP600125) and Akt (Akt inhibitor III) did not block UVB induced increase in c-kit. However, inhibitor of p38 (SB203580) blocked the UVB-induced increase the c-kit expression and partly inhibited the phosphorylation of MITF. Combined results suggest that UVB-induced increase in c-kit expression is mediated through p38 and once MITF is phosphorylated in part through p38 pathway then activated MITF would also participate in increasing the expression of c-kit.

Rouzaud et al. from NIH and U. Cincinnati College of Medicine examined how different isoforms of MC1R are regulated. Normally MC1R with amino acids numbers of 317 (MC1R317) is expressed on melanocytes. This group has identified MC1R 350, alternatively spliced form of MC1R317. Interaction with ^{125}I-α-MSH was similar between two isoforms of MC1R. The melanin content correlated with MC1R317 level but inversely correlated with MC1R350 level. Over-expression of MC1R317 into melanocytes caused increase in tyrosinase expression where as over-expression of MC1R350 dampened the expression of tyrosinase. Results suggested that darker skins may have more of MC1R 317. Understanding of MCIR350 regulation may provide further insights in biology of melanocytes.

Bellei et al. from NIH presented results on how β-catenin is degraded after UVA irradiation. UVA-irradiation of normal human melanocytes (8-16 and 32 J/cm^2) decreased E-cadherin expression where as the expression of N-cadherin and α-catenin remained unchanged. There was a slight down-regulation of β-catenin and cytoskeleton was reorganized. Fractionation of cell lysates revealed that β-catenin degradation primarily occurs in the cytoplasm, in the cytoskeleton and in the nucleus but not in the membrane fraction. Caspase 3 is the major protease responsible for the degradation of β-catenin

Schepsky et al. from U. Iceland, U. Freiburg and UK reported that when MITF interacts with β-catenin then the transcriptional complex is directed toward MITF specific genes. In a yeast two-hybrid system, β-catenin co-immunoprecipitated with ^{35}S-MITF and LEF-1. Over expression of MITF reduced β-catenin-dependent gene expression. Conversely, over-expression of β-catenin enhanced MITF-dependent gene expression. Therefore, MITF sequesters β-catenin away from the β-catenin target genes to MITF target genes.

Schuierer et al. from U. Regensburg examined RKIP expression in melanoma cell lines. RKIP (raf kinase inhibitor protein) is the physiological inhibitor of raf signaling pathway. It is increased in nevus, decreased in primary melanoma and dramatically reduced in metastasized melanomas. To determine mechanisms through which the expression of RKIP is reduced in melanomas, they examined hypermethylation of RKIP gene since RKIP promoter regions contained number of CpG islands. However, treatment of cells with demethylation agents did not change RKIP expression. Transcription repressor Snail decreased the expression of RKIP. The stability of RKIP mRNA was also altered. Combination of transcription and mRNA stability contributes to the loss of RKIP in melanoma cells.

Ivanova et al. from Germany and Amsterdam University presented results that nitric oxide (NO) induces detachment of melanocytes through cGMP pathway. When normal melanocytes and vitiligo melanocytes were treated with NONOates, both types of melanocytes displayed detachment in the dose-dependent matter. Treatment with apoptosis inhibitor showed that the major part of detachment involved apoptosis. The NO-induced detachment was partly inhibited by cGMP inhibitor but not the apoptosis. Therefore, the NO may utilize cGMP pathway to detach melanocytes from extracellular matrix proteins such as fibronectin.

Smit et al. from Netherlands presented results on role of calcium/calcineurin/NFAT pathway in melanocytes. Calcineurin is a calcium/calmodulin dependent protein phosphatase. When this pathway is activated NFAT is translocated to nucleus and activates transcription. TPA activates this pathway. By comparing the genes affected by this pathway using microarray before and after TPA treatment and comparing between normal human melanocytes and melanoma indicate that genes are differentially regulated.

Seiberg et al. from Johnson and Johnson presented data that peptides SLIGRL, LIGL and RL induced skin darkening. All three peptides increased GTP-Rho activity and cytoskeleton reorganization. However, while SLIGRL stimulated ERK1/2, p38, AKT and IKB, LIGR did not induce phosphorylation of these kinases. They conclude that the shorter peptides LIGR and RL activate only a subset of the PAR-2 signaling pathways. These smaller peptides may be more desirable for skin pigmentation since limited pathway maybe activated, thus minimizing side effects.

Concurrent Session 4 – "Innovative Technology"

By Miri Seiberg

The innovative technology session provided four exciting talks on very different technologies; all have the potential to be used by many investigators in the future.

Dr. Kachhawa from the Medical College of Jodhpur, India, presented a comparative study of epidermal transfer techniques for the treatment of vitiligo. His novel technique, ECTT, was shown to be simpler and superior to the standard SGT (split thickness graft) technique. ECTT is an epidermal cell transfer technique where donor site is dermabraided, and the collected deeper layer- skin fragments are applied onto dermabraided vitiligo sites and bandaged. ECTT requires local anesthesia

only, results in less damage and faster recovery of the donor sites, is suitable for large treatment areas, is less expensive and labor intense relative to SGT, and results in superior cosmetic results.

Dr. Kobayashi presented a collaborative research of three Japanese groups, on the internalization of c-kit in a clathrin-independent way. C-kit, an important player in melanocyte survival and migration, has been shown earlier to be internalized via a clathrin-dependent pathway. Using ligand-stimulated receptor assays, the team showed tyrosinase phosphorylation with 50% of c-kit remaining active at 10 minutes post stimulation, while internalization was documented as early as after 2 minutes. Localization studies showed that most of the activated c-kit was inside the cell. A hand held laser excitation system was presented, which was coupled to microscopy, and enabled visualization of GFP-conjugated proteins at the single molecule video imaging level. Using this system with clathrin pathway blockers or mutants, the early internalization of c-kit was documented, suggesting a clathrin-independent pathway that acts earlier than the clathrin-dependent pathway of c-kit internalization.

Dr. Yajima of Institut Curie, France, presented a transgenic system that enables to activate or inactivate a candidate gene in the melanocyte lineage at any time. A Cre-LOX system with an inducible tyrosinase promoter was used to create a transgenic line, when tyrosinase is coupled to an estrogen promoter and is activated by Tamoxifen. Titration of Tamoxifen dose and time was performed to avoid toxicity, and results were demonstrated by lac-z expression. Induction was demonstrated in vivo both in immature and mature melanocytes, with some ectopic expression in non-melanocyte cells. This transgenic system provides a research tool for identifying the role of candidate genes during melanocyte development and transformation.

Eduardo Ruvolo presented a collaborative study of Indian clinical facilities with the Johnson & Johnson imaging team. The study developed documentation of characteristics of melasma in Indian population using image analysis, and correlates these findings to clinical evaluations, using MASI scores. An integrated imaging system with an immobilized chin position, used polarized and cross-polarized lights to create images for lesion documentation, to enhance lesion borders and to measure darkness of lesion and no lesion sites. Diffuse reflectance spectroscopy documented the contribution of pigment, hemoglobin and deoxy-hemogblobin and dermal scattering to the visualized lesion color. A correlation with MASI scores was established, suggesting that this imaging system could be used as an objective tool to document melasma progression over time or treatment.

Concurrent Session 5 - " Melanosome Structure and Function"

by Vijayasaradhi Setaluri

That the biology of melanosomes is here to stay as major topic of pigment cell biology is demonstrated by its representation at the 19th International Pigment Cell Conference in a Plenary Symposium (Melanosome Biogenesis, Motility and Transfer) and a Concurrent Session (Melanosome Structure and Function). In addition to many excellent poster presentations, there were a total of ten oral presentations in these sessions. At the concurrent session chaired by Drs. Borovansky (Charles Univ., Czech Republic), Kidson (U. Cape Town, South Africa) and Setaluri (U. Wisconsin, USA) four abstracts on various aspects of melanosome structure and function- ranging from early events in melanosome biogenesis to factors that determine the fate of melanosomes transferred to keratinocytes- were presented.

It is becoming increasingly clear that despite shared sorting signals melanosomal membrane proteins follow different intracellular pathways to arrive at the melanosomal membrane. For example, the pathways that TYRP1 follows appear to be distinct from that followed by tyrosinase. Consistent with such notion, Vijay Setaluri's laboratory had earlier shown that newly synthesized TYRP1 interacts specifically with a cytoplasmic PDZ domain protein GIPC, suggesting a role fro such interaction in TYRP1 sorting. However, the exact site of interaction and the mechanisms of action of GIPC remained

to be investigated. Kedlaya et al. (U. Wisconsin, USA) in their presentation showed data that suggest that TYRP1-GIPC interaction occurs at the endoplasmic reticulum (ER) and that this interaction is required for efficient export of TYRP1 from the ER. Additionally, using a combination of biochemical and cell biological methods, they also show that oligomerization of GIPC molecules, presumably on the cytoplasmic face of ER, is required for its action. They suggested that GIPC may cluster newly synthesized TYRP1 molecules on the ER for vesicular export similar to function of PDZ domain containing proteins in clustering cell surface receptors. After they are exported to *trans*-Golgi, a complex set proteins are required in orchestrating the assembly of melanosomal proteins into melanosomes. The discovery that defects in the biogenesis of lysosome-related organelles underlie a spectrum of human hypopigmentary disorders known as Hermansky-Pudlak Syndrome, made melanocytes derived from these patients a valuable cellular reagents for detailed investigation of events in melanosome biogenesis. In their presentation Helip-Wooley et al. showed that in adaptor complex-3 (AP3) defective HPS-2 cells, trafficking of tyrosinase but TYRP1 is affected. Additionally, these studies in Dr. Bill Gahl's laboratory (National Institutes of Health, USA) showed that the clathrin-binding domain protein HPS-3, which is defective in patients of the subtype HPS-3, binds to and colocalized with clathrin, and that abnormal distribution of melanosomal proteins in HPS-1 and HPS-5 melanocytes can be corrected by expression of respective proteins. Although it is generally known that melanin biosynthesis closely resembles catecholamine metabolism and that melanocytes can synthesize catecholamines, it is not known whether tyrosinase plays a role in catecholamine metabolism in melanocytes. Data presented by Matsunga et al. (Tohoku University, Japan) suggested that notwithstanding its localization within the lumen of melanosomes, tyrosinase also controls cytosolic catecholamine metabolism. They showed that treatment of B16 melanoma cells with phenylthiourea enhanced the cytotoxic effect of dopamine (DA), but not L-DOPA, on these cells. This implicates a role for localization of tyrosinase in detoxifying DA. Matsunga et al. also reported that consistent with this requirement of transport of cytosolic DA into melanosomes, B16 melanoma cells express dopamine transporter (DAT) and it is localizes to lysosomes (and melanosomes?). While discovering novel function for melanogenic enzymes and melanosomes within melanocytes continues, factors that determine the patterns of distribution of melanosomes exported into keratinocytes have largely remained a mystery. This latter topic was the focus of Yoshida et al. Earlier cell culture observation by Dr. Raymond Boissy' laboratory (U. Cincinnati, USA) showed that distribution of melanin pigment within keratinocytes is determined not by factors intrinsic to the melanocyte or melanosome but by the recipient keratinocyte. Expanding on this, again Dr. Boissy's group elegantly demonstrated this intrinsic function of keratinocytes in an in vivo human skin substitute system. First, they showed that the reconstituted keartinocytes, melanocytes and fibroblasts produce a skin substitute graft, on the backs of SCID mice, that recapitulates the pigment type of the donor skin. Then by mixing keratinocytes and melanocytes from different skin types they showed the pigment distribution in keratinocytes is determined by the pigment type of the skin from which keratinocytes.

Workshop – "Genetics and Developmental Biology"

by William J. Pavan

There was a joint workshop encompassing both the Developmental Biology & the Genetics interest Groups of the IFPCR. This workshop was hosted by Robert Kelsh, Hiroaki Yamamoto and Bill Pavan. The session began with an invited lecture by Keith C. Cheng, M.D., Ph.D, Associate Professor of Pathology, Biochemistry and Molecular Biology, and Pharmacology, Jake Gittlen Cancer Research Foundation Penn State College of Medicine. His presentation titled "Unexpected insight into human skin color from golden zebrafish" described recent studies involving the positional cloning of the gene mutated in the zebrafish mutant, *golden*. They found that the mutation resides in a member of the potassium dependent sodium, calcium exchanger gene family. Interestingly his group also went on to propose that polymorphisms associated with this gene may account for a large component of the genetic contribution to skin color variation in man.

Following the invited talk, four more talks were scheduled. Dr. Cooper from the University of Washington, Seattle, USA described evidence that Foxd3 and c-kit signaling cooperate to regulate melanogenesis. They propose that this may act through regulation of MITF transcription. Dr. Hou from the National Institutes of Health, Maryland, USA described a series of experiments to determine if MITF could rescue melanocyte differentiation in SOX10 mutant neural crest. He proposed that MITF alone is not sufficient to completely replace the need for SOX10 in mammalian melanocyte development. Dr. Kawa of St. Marianna University School of Medicine, Kawasaki, Japan, described experiments examining melanocyte differentiation in primary neural crest explant cultures from MITF mutant mice and by knockdown of Mitf in NCCmelb4M5. Results from her experiments suggest that Mitf plays a role in melanocyte survival in early developmental stages. Dr. Akiyama of Dept. Biology, Keio University, Yokohama, Japan described recent work on the Silky chicken which shows heavy pigmentation on internal organs. She proposes that increased signaling through the endothelin pathway early in melanoblast migration may be responsible for the increased melanization seen in these animals.

Tuesday, September 20, 2005

Sunrise Session 2 – "Differentiated Functions of Melanocytes

by William A. Gahl

Dr. Stefana Petrescu laid the groundwork for the day's talks by discussing how melanocytes perform their differentiated function, i.e., the production of melanin. The pathway requires melanogenic enzymes and structural proteins interacting within the melanosome. These subcellular organelles mature structurally and biochemically as they move centrifugally to the dendritic tips, where they are transferred to keratinocytes, providing pigment to hair, skin, and eyes. The copper enzyme tyrosinase plays the initial, crucial role in the synthesis of both eumelanin and pheomelanin; the enzyme's egress from the endoplasmic reticulum is modulated by calnexin, a protein that fosters proper folding of tyrosinase molecules. Glycosylation is critical for folding, as demonstrated by aberrations due to glycosylation mutants; some OCA I mutations are defective in folding. Tyrosinase undergoes processing in the Golgi and eventually reaches the melanosome. Other melanogenic proteins include Tyrosinase-related Protein 1, Tyrosinase-related Protein 2, Pmel17, P, the OA1 receptor defective in ocular albinism, and Mart-1. Melanosomes mature as they move along microtubules for large distances and actin filaments for shorter distances, laterally near the nucleus and plasma membrane. Kinesins serve as motors binding a melanosome to the microtubule, and rab GTPases (e.g., rab27a) provide energy; when transfer to actin filaments is needed, specific proteins such as melanophilin connect with myosin Va on the actin filaments. The different types of albinism represent defects in various stages of the pathway described above. Hermansky-Pudlak Syndrome (HPS) represents a defect in the formation of lysosome-like organelles, which include not only melanosomes, but also platelet dense bodies and subsets of lysosomes. Hence, HPS patients have variable degrees of hypopigmentation plus a bleeding disorder. Seven different genes have been identified to cause autosomal recessive HPS; all are considered to be involved in vesicle (i.e., melanosome and platelet dense body) formation and trafficking, but only the β3A subunit of adaptor complex-3 (whose deficiency causes HPS-2) has a defined role in this process. Chediak-Higashi patients' granulocytic cells display giant granules due to aberrant vesicle trafficking, and affected individuals suffer life-threatening infections, the hemophagocytic syndrome, and late neurological involvement. Griscelli syndrome patients have silvery hair and can have neurological impairment or the hemophagocytic syndrome. These inborn errors of pigment formation provide insights into the normal processes of melanosome formation and movement.

Plenary Symposium 6 – "Differentiated Functions 1; Differentiated Functions of Melanocytes / Melanophores (Oculocutaneous albinism).

by Francisco Solano

The session was opened by Prof. Richard King, one of the 3 co-chairs, and the first Plenary Lecture was given by Prof. Tomita, from Nagoya University, with the title “Oculocutaneous albinism type 4 is one of the most common types of albinism in Japan”. Prof. Tomita introduced this lecture with basic definitions about what oculocutaneous albinism (OCA) is, its different types, and some historical facts. The first reported cases of OCA in human being were tyrosinase-negative, but pioneer work from Prof. Witkop introduced other types of OCA patient showing positive tyrosinase activity. The discover and characterization of other proteins essential for pigmentation allowed for a better classification, relating OCA2 to P protein, OCA3 to Tyrp1 and OCA4 to MATP. This last one is a serpentine protein with 12 transmembrane fragments found in the melanocyte membrane whose biochemical function is still not well-known, although it seems to be related to saccharide transporters, probably Glu-4 type.

Moving to statistics, he presented an updated list of Japanese patients with clinical phenotypes, doing special emphasis in OCA4, which in Japan is almost as frequent as OCA1 (from a total of 75 albinism patients, 38 were OCA1 but 30 were OCA4). The severity of the symptoms is variable. The most common mutation is the D157N. He finally established some comparison to OCA4 in other countries, such as the cases described in Germany.

The second plenary lecture was given by Dr Jimbow, from Sapporo Medical University, with the title “Melanogenesis cascade and biology of normal and abnormal pigmentation”. Prof. Jimbow offered to the audience a complete overview of melanogenesis mechanisms at cellular and molecular levels in 4 key steps. Most of the work he presented has been carried out in his wide research career, including (a) the study of proliferation of stem cells and the role of c-kit and endothelins, (b) the folding of tyrosinase and the use of castanospermine to explore tyrosinase maturation, interaction with chaperones and final activity, (c) the transport of tyrosinase and its related proteins to melanosomes and the crucial role of Rab7 in this vesicular process, and (d) assembly of tyrosinase and other melanosomal proteins to form the melanogenic complex and the final deposition of melanin, emphasizing the protective role of Trp1 as a protein which rescue melanocytic cells from tyrosinase-mediated cell death and in summary the synergistic role of Trp1 and Trp2 on tyrosinase melanogenic activity.

As scheduled, after the two plenary lectures, four communications were briefly orally presented. These communications were previously selected by the organizing committee on the basis of their high scientific interest. The first one was presented by Dr. Murray Brilliant with the title “Gene polymorphism and human pigmentation”. His work tried to know the relative contributions of the main genes encoding melanin-related proteins to pigmentation of hair, eyes and human skin color. It is very likely the most extended study never made, involving 800 participants including African, Asian and European individuals genotyped at 48 polymorphisms in 17 genes. Polymorphisms in five genes, P protein MATP, MC1R, ASIP and DCT, account for 60-80% of the variance in skin and hair pigmentation. For eye color (blue, green, hazel, gray, brown, black), these genes account for only 39%, indicating that eye color is genetically the most complex pigmentation trait. Perhaps some other candidates, as Pmel17 or some Na+/H+ changers, should be added to the study. According to this data, in the near future it would be possible to predict the skin and hair color from a DNA sample by genotyping only a few polymorphic loci, with clear interest in forensic medicine and criminal investigation.

Secondly, Dr. S. Petrescu presented data about the traffic and maturation of tyrosinase, the key enzyme for melanogenesis. Some OCA 1 are related to changes in the hydrophobic region of this enzyme. She presented data using tyrosinase mutated at its C-terminal tail (soluble tyrosinase) to investigate the maturation process and the role of this fragment in the ER. Basically, she showed using this elegant approach that truncated tyrosinases are retained in the ER to be degraded through the proteasomal pathway. Interestingly, this form of tyrosinase interacts with BiP and calreticulin but not with calnexin

as the native form of the enzyme does. However, N-glycosylation and mannose trimming is required for the degradation process. She defined soluble tyrosinase as an ERAD (Endoplasmic Reticulum Associated Degradation) substrate. All together, Dr. Petrescu demonstrated that mutations in the C-terminal region of tyrosinase cause a change in the specificity of chaperones interacting to the enzyme, giving calreticulin a novel role in these particular cases of OCA 1.

Thirdly, Dr. G.E. Costin reported some data about the effect of dopachrome tautomerase activity in the eumelanin/pheomelanin ratio in mouse melanocytes. Using C57Bl/6J non-agouti melanocytes and two well characterized Dct-mutant cell lines, *slaty* and *slaty light*, she showed that Dct activity is decreased approximately 3-fold and 28-fold respectively. Chemical analysis showed that both mutations increase pheomelanin and reduce eumelanin produced by those melanocytes in culture, in comparison to the non-agouti black phenotype. In turn, her results also demonstrated that the above mentioned mutations do not affect intracellular trafficking of the respective mutant proteins, but modelling studies indicate that the first mutation (R194Q) is located near or at the active site to alter the affinity of the enzyme for the substrate, and the second one (*slaty light*, G486R) may result in the sliding of the transmembrane domain towards the N-terminus of mutant Dct, affecting Dct function probably in the eumelanin complex. Taken together, the level of Dct activity seems to play a role in determining the preference for the eumelanin pheomelanin pathway in pigment biosynthesis.

Finally, Dr. Montoliu presented an interesting study under the title “Gene expression profile analysis in normal retinal development in mammals: a comparative approach between albino and pigmented animals”. Albino patients (OCA 1) undergo some visual anormalities related to low number of photoreceptors and abnormal cellular connexions between retina and brain. The molecular mechanisms and interactions between lack of tyrosinase and appearance of these abnormalities are unknown. Dr. Montoliu presented that the defects can be mostly corrected in animal albino model inserted with functional tyrosinase transgene, indicating the role of tyrosinase gene product(s) in retina development. As very novel data on genes possibly related to albino abnormalities aside pigment absence, Dr. Montoliu discussed the possible role of Tia-1 overexpression in albino mice. This factor is overexpressed as a stress response in hypoxia, and he showed that tyrosinase induction reduces Tia-1 expression in albino background.

All presentations were followed by some brief questions and discussions on the particular aspects raised by the audience. A great number of researchers attending to the meeting participated in those minutes, with the only limitation of the time scheduled for this session.

Plenary Session 8 - “Regulation of Pigmentation” Regulation of Melanocyte/Melanophore Function (constitutive pigmentation/environmental responses)

by Richard M. Niles

“Defining the Role of Melanocortins and the Melanocortin 1 Receptor in Preserving Human Melanocyte Survival and Genomic Stability” Zalfa Abdel-Malek

There are extensive polymorphisms within the melanocortin-1 receptor (MC1R) gene. Some these are associated with red hair, poor tanning ability and increased susceptibility to melanoma. Dr. Abdel-Malek’s lab found that these particular alleles of the MC1R gene represent loss-of-function mutations in the MC1R gene. They render human melanocytes refractory to melanocortins and disrupt their normal response to UV radiation. Recently the lab has focused on the role of alpha-MSH as a survival factor that rescues human melanocytes from UV-induced apoptosis. This pathway involves, activation of Akt, increase in totl and phosphorylated of MITF and increased levels of Bcl2. DNA repair enzymes were increased, while ROS was decreased in alpha-MSH- treated cells. These effects were not seen in human melanocytes having loss of function MC1R genes. The lab is currently investigating the UV-radiation response in human melanocytes using DNA microarrays.

"Transcription and Signaling in Melanocytes and Melanoma" Colin Goding
The lab recently published that increased levels of MITF can inhibit proliferation of some melanoma cells. However, Dr. Goding showed some unexpected results, that knock-down of MITF also led to decreased cell proliferation. This was accompanied by a decrease in the cyclin-dependent kinase inhibitor p21 and increase in p27. MITF knock-down cells also have a change in their shape, due to an altered distribution pattern of actin filaments.
Dr. Goding concluded his talk by presenting a new model for the role of MITF. In this "rheostat" model, the effect of MITF on growth and survival depends on the state of the melanocyte (melanoblast, mature melanocyte, stem cell, melanoma) and the relative level of MITF present in these cells.

"Upstream Stimulating Factor (USF-1) A Potent Stress-Response Transcription Factor", MD Galibert
USF1/2 are conserved transcription factors of the bHLH-leu-zip family. They bind to E-box DNA elements in target genes and recruit chromatin remodeling enzymes, interact with coactivators and members of pre-initiation complexes. Their transcriptional activity is dependent on post-translational modification. The lab previously found that USF-1 is phosphorylated by p38 MAPK. They showed that the tanning response involving the POMC, MC1R, Tyrosinase. TRP-1 and Dct genes is dependent on p38 activation of USF-1 initiated in response to UV-radiation-induced stress. Dr. Galibert recently discovered a second post-translational modification, termed M-USF-1, that occurs in response to distinct stress signals such as UV radiation, viral infection, etc. M-USF-1 binds to E-box DNA as demonstrated by ChIP, but it could function as a dominant-negative regulator.

"Genetic Models of Human MC1R Variant Receptor Alleles for Pigmentation Phenotype and Cellular Function in Signal Transduction", Rick Sturm
The lab has quantified the contribution of individual MC1R alleles to pigmentary phenotypes in a large family of adolescent twins, partents and sibling. Four common and two rare alleles termed large R were strongly associated with red hair and fair skin. An additional 3 alleles designate small r had lower penetrance compared to the WT allele. Recent studies have examined the functional ability of MC1R variants to activate the cAMP pathway in transfectants of HEK293 stably expressing the gene. R associated variants showed agonist-induced cAMP levels and CREB phosphorylation. One of these variants, (D294H) showed severe impairment of the functional response. Cumulatively, these data indicate that these alleles are not complete loss of function receptors and are not equivalent. Additional studies examined the subcellular localization of the R receptors. Found that melanocytic cells expressing endogenous or ectopic receptor exhibited strong surface localization of WT and the D294H alleles, but markedly reduced surface expression of R151C and R160W R alleles. The r allele encoded MR1C receptors had normal or intermediate cell surface receptor levels.

"Melanocortin 1 Receptor Dimerization: Functional Consequences and Dominant-Negative Effects", J.C. Garcia-Borron
Through the use of co-immunoprecipitation of differentially epitope-tagged MC1R forms, dimeric and oligmeric forms of MC1R species were discovered. Dimerization occurred early during MC1R synthesis as revealed in studying MC1R mutants displaying intracellular retention and decreased plasma membrane expression. These mutants exerted a dominant-negative effect on WT MC1R. On the other hand, partial complementation of selected loss-of-function mutants was observed. WT MC1R did not exhibit this cooperativity, but co-expression of WT MC1R and a C-terminal deletion mutant yielded a form with higher affinity for agonist binding, but lower coupling efficiency than WT. Common natural diminished function alleles associated with red hair and increased melanoma risk, were able to heterodimerze with WT MC1R. These results suggest that specific combinations of MC1R are associated with subtle changes in MC1R functional properties, indicating that the presence of mutant MC1R alleles may have consequences beyond those based on dosage effects and haploinsufficiency.

"Shorting and Trafficking of PMEL17 (GP100): Evidence for the Polarized Nature of Melanocytes", J.C. Valencia
Pmel is a constituent of the melanosomal matrix in melanocytes. It is also a target for immunotherapy in patients with melanoma. The lab has characterized the processing and trafficking of Pmel 17 via AP complexes. Pmel 17m AP1 and AP2, but not AP3 or 4 were detected in early melanosomes. Two forms for AP1 (AP1A and AP1B) are involved in epithelial cell-specific complexes involved in polarized sorting to the plasma membrane. The presence of AP1B in human melanocytes was confirmed by Q-RT-PCR, immunolabeling and in situ hybridization. Transfection of AP1 isoforms shown both a central area distribution for AP1A and a peripheral distribution for AP1B. AP1B is expressed in early stages of melanoma, while metastatic cells loose expression. Pmel17 is sorted to the plasma membrane regardless of AP1B expression. The results of this study suggest that Pmel17 is sorted to the melanosomes directly or indirectly through the plasma membrane. Presence of basolateral elements in melanocytes suggest the polarized nature of the melanocyte and suggest that loss of this polarization may be involved in malignant transformation and metastasis.

Concurrent Session 6 – "Comparative Biology"

by Manfred Schartl and Manickam Sugumaran

The Concurrent Session "Comparative Biology" was composed of four presentations: (1) Diversification of melanin pigmentation caused by transposable elements in Medaka fish, (2) The Xmrk (Xiphophorus melanoma receptor kinase) is sufficient for induction of melanocyte migration, (3) Co-purification of DOPA chrome isomerase and quinone isomerase isolated from Calliphora and (4) A search for genetic determinants of color variability in the panther chameleon.

The first topic was presented by M. Koga who has a long career on studies of transposons. He showed clear involvement of this element as one of the key steps yielding a wide variety of color mutants in Medaka fish. The juveniles of i b albino of this species are installed with small and varying sized melanophores, as a result of insertion of the Tol2 transposon in the promoter region of its tyrosinase gene. In as much as this DNA-based transposon moves within the gene in a cut-and-paste manner, the complete excision of this element from the tyrosinase gene is expected to recover the wild type phenotype. Even though this transposon is known to be inserted in particular positions of the gene, it leaves different footprint sequences behind upon excision, thus yielding various phenotypes with respect to melanin pigmentation. Koga indicated that the newly arising alleles are inherited in the Mendelian fashion and, at discussion, that in such revertants, pigmentation is similar between skin melanophore and pigment epithelium.

The second presentation was presented by M. Schartl who has first clarified the biochemical structure of the so-called "tumor gene" in platyfish, which was termed Xmrk later and is a bonafide oncogene by modern definition. The over-expression of Xmrk, a receptor tyrosine kinase of the EGFR family, is considered to be the critical step for the initiation and progression of melanomas in inter-species hybrids of the genus Xiphophorus through the signaling pathway depending on PI3 kinase, STAT5 and Ras/Raf/MAPK. Xmrk dependent signaling is responsible for increased proliferation, dedifferentiation and protection against apoptosis in 3D collagen matrices of melanoma cells. Based on these findings, Schartl further examined the signaling pathways associated with migratory behaviors of Xmrk transformed mouse melanocytes (melan-a) using 2D and 3D migration assays. The migration of these cells was shown to be dependent on the activities of the focal adhesion kinase FAK and the non-receptor tyrosine kinase fyn but not on the Ras/Raf/MAPK pathway. Thus, it becomes apparent that Xmrk induces migratory behavior characteristic to neoplastic phenotype in them through its signal transduction. In the second part of his talk Schartl presented a transgenic melanoma model in Medaka. Using the pigment cell specific MITF-promoter to drive Xmrk expression, transgenic Medaka develop pigmentation abnormalities ranging from hyperpigmentation to melanoma. In the discussion, the

possibility that the MITF promoter in lower vertebrates functions equally in melanophore and in the xanthophore/erythrophore lineage was brought up.

The third topic was presented by M. Sugumaran, whose expertise is concentrated to the chemistry of phenoloxidase in arthropods. The hot issue of this presentation is the possible presence of a novel protein in Calliphora, which possesses the activities of both dopachrome isomerase and quinone isomerase. Thus far, extensive similarities are strongly suggested with regard to the biochemical transformation of melanization and sclerotization pathways in these organisms. A good example is given by quinone isomerase present in the sclerotinogenic pathway, since this enzyme converts N-acetyldopamine quinones formed by phenoloxidase to quinone methides as the dopachrome isomerase in mammalian melanogenesis. When the quinone isomerase is isolated from the hemolymph of this organism through various protein purification protocols, the dopachrome isomerase activities are also recognized together. Based on these findings, it is postulated that either these two activities are present on the same polypeptide chain or two different proteins are bound together. He emphasized that this observation should provide a chemical basis for a dual role of arthropod phenoloxidase in both melanization and sclerotization.

The final talk was given by R. L. Morrison who is attracted by the spectacular variation of pigmentary phenotypes in Madagascan Panther chameleon. This species is sexually dimorphic and the male vary heritably in skin pigmentation from one habitat to another, being predominantly blue in some localities (Nosy Be) whereas predominantly orange in others (Tamatave) with vertical bands of different combinations of blue and red or green and blue or green and orange etc. Some variant in another place (Ankaramy) has red spots on a pink background. In as much as skin coloration of these chameleons is formed by a very thick layer of five distinctive types of chromatophores arranged in a specific pattern within the upper layer of the dermis, it is expected that such variation in pigmentation of this species may be associated with specific polymorphisms in the melanocortin 1receptor (MC1R) as observed with mammals, birds and reptiles. Search has now been made for a genetic basis determining locality-specific color patterns. At discussion, the possibility of diet effects was pointed out but denied based on the fact that all variant chameleons under captive are fed similarly with crickets.

The board of chairpersons, M. Schartl, M. Sugumaran and J. Matsumoto, was very much pleased with a large audience and active discussions.

Concurrent Session 7 – “Chemistry and Physics of Melanins

by Kazumasa Wakamatsu

Dr. L. Hong of Duke University presented regarding the morphology and photoionization potential (IP) of RPE melanosomes and ocular lipofucin granules. Bovine and human RPE melanosomes were measured with SEM, AFM and photoelectron emission microscopy (PEEM). These morphology properties of these samples varies with tissue, age of samples and species. The IP of the samples were also determined using PEEM. Examination of single bovine RPE melanosomes suggests no correlations between the photoionization potentials (IP) and shape of melanosomes. They indicated that PEEM of two human lipofuscin samples (14-year and 76-year) reveal two IPS and the ratio of these two components was found to change with age.

Dr. Pezzella of Naples University presented on a new approach to the study the process of polymerization of 5,6-dihydroxyindoles (DHI) to melanin pigments, based on oxidation of the main dimers obtained by oxidation of the indoles under biomimetic conditions. In this way a restricted number of higher oligomers were isolated and characterized with respect to the structure and other properties, e.g. chiraloptical properties of 5,6-dihydroxyindole-2-carboxylic acid tetramers, providing new vistas on melanin pigments. The attention was particularly focused on the isolation and characterization by extensive 2D-NMR and mass spectrometry of a tetramer obtained by

peroxidase/H_2O_2 oxidation of the 2,4' DHI dimer. The results of a pulse radiolytic investigation of the oxidation of 2,4' and 2,7' dimers of DHI carried out at Daresbury Laboratories in collaboration with Dr. Land were also presented. Evidence was obtained for the formation of two different semiquinone species for each dimer, which decayed with second order kinetics to quinonoid product(s) exhibiting absorption maxima at 530 and 550 nm. The symmetric 2,2' dimer on the other hand afforded a single semiquinone disproportionating at a much slower rate to give a quinone with absorption maximum at 580 nm, that is bathochromically shifted compared to those of the other dimmers. This would point to a better conjugation of the indole units in this latter quinonoid species. In the discussion that followed the presentation the mechanisms of the polymerization reaction were debated and some suggestions were given based on consideration of the structure of the oligomers so far isolated.

Dr Napolitano of Naples University presented the specific marker of pheomelanin given on the chemical degradation method. Red hair, fair skin and lack of tanning, which are associated with some loss of function mutations at the melanocrtin-1 receptor (MC1R), are recognized as risk factor for melanoma and other skin cancers. The loss of functions of MC1R cause the melanocyte to produce the pheomelanin. However, the similar red haired individuals do not exhibit the same erythemogenic responses and tanning capacities. This suggests that pheomelanin variants with different photoprotective and/or photosensitizing properties exist. On the chemical degradation of pheomelanin they used 1,3-thiazole-2,4,5-tricarboxylic acid (TTCA) and 6-(2-amino-2-carboxyethyl)-2-carboxy-4-hydroxybenzothiazole (BTCA) as the pheomelanin marker. From the data of 22 red haired individuals, BTCA was main product in the lowest MED and 5-days delayed pigmentation, while TTCA in higher MED values (mean value 67.5 mJ/cm^2, $p < 0.001$). As a result, they suggested that the quantification of these markers would give the potent mean for routine prediction of high risk individuals.

The presentation by Dr. Ito with Drs. Wakamatsu, Kanavagh and Abdel Malek as coauthors was focused on the analysis of primary human melanocyte cultures established from 49 individuals (42 neonatal and 7 adult) exhibiting significant diversity of visual pigmentation. Identification and quantification of the typical eumelanin and pheomelanin markers, pyrrole-2,3-5-tricarboxylic acid (PTCA) and 4-amino-3-hydroxyphenylalanine (4-AHP), by the chemical degradation of melanocytes allowed to determine the eumelanin and pheomelanin content for each melanocyte culture. The melanin content was also determined spectrophotometrically and the data on melanin pigmentation were correlated with the tyrosinase levels and activity as determined by the Pomerantz assay and with the mutations of MC1R gene. Data presented in the communication showed a good correlation between the spectrophotometric total melanin content and eumelanin plus pheomelanin content as determined by chemical degradation and between these data and the visual phenotype. Also, the eumelanin content showed a positive correlation with the levels of tyrosinase, while the relationship of the pheomelanin content with the visual phenotype was not straightforward.
A most interesting aspect which was highlighted is that some MC1R loss of function mutations do not show a clear cut correlation with the chemical phenotype, hence mutations at this gene do not apparently alter the phenotype but may significantly affect the sensitivity of melanocytes to UV-induced damage. The discussion that followed the presentation pointed out aspects related to chemical analysis of melanin pigmented tissues and the possibility to introduce, in addition to the genotype, a "chemotype" that is a classification of the pigmentary pathways as determined by analysis and quantification of the resulting pigments was also considered.

Concurrent Session 8 – "Photobiology"

By Helene Z. Hill

Four papers were presented in this session that dealt with various aspects of exposure to mixed wavelength UV from solar simulators versus UVA. Experimental subjects ranged from cultured cells to human volunteers. Dr. Marrot from L'Oréal started off by describing the stress responses observed in cultured Caucasian melanocytes after exposure to solar simulated radiation (SSR) and UVA

delivered at sub-lethal doses. The melanocytes arrested at G2/M with concomitant accumulation of GAD45 and XPC. Induction of p53 was less in the melanocytes than in fibroblasts. Heme oxygenase-1 was upregulated in melanocytes following both types of irradiation particularly if pigmentation had been induced.

Dr Yamaguchi from the National Cancer Institute and colleagues looked at DNA damage in the form of pyrimidine dimers and 6-4 photoproducts, apoptosis and phosphorylation of p53 in African-American and Caucasian skin. Both types of DNA damage were similar in upper epidermis but reduced in lower epidermis in African-American skin. Furthermore, apoptosis was greater in the dark skin. These findings suggest that in more heavily pigmented skin, both protection against DNA damage and efficient removal of damaged cells are responsible for the lower rates of skin cancer in darker skin.

Dr. Briganti from Rome and his colleagues studied the interaction of pheomelanin and gap junction intercellular communication (GJIC) in cultured human keratinocytes. GJIC were reduced 2 hours following UVA exposure but were restored by 6 hours. When pheomelanin was present during the irradiation, the effects were enhanced. Induction of GSH in the cells before irradiation abrogated the effects, while depletion of GSH led to enhancement. It was concluded that pheomelanin can interfere with GJIC during sun exposure and high or repeated exposures could lead to long term effects on GJIC.

In the final talk by Dr. Wolberg from Hamburg, Germany, UVA responses were compared to those of a solar simulator (SSR) in suction blisters from volunteers. The two types of irradiation had dramatically different effects with SSR producing up regulation of tyrosinase, TRP1, MITF and p53 which were not seen after UVA although both types of irradiation resulted in tanning apart from IPD and PPD. It is clear that responses to solar radiation designed to model natural exposure similar to holidays at the beach are quite different from responses that would result from UVA sun bed exposure.

Wednesday, September 21, 2005

Sunrise Session 3 – "Pigmentary Disorders #2"

by Proshiela Manga

Normal skin pigmentation is contingent upon four criteria. 1. Migration of melanoblasts from the neural crest to the epidermis and differentiation of these precursors to melanocytes. 2. Pigment synthesis in melanosomes. 3. Melanin transfer to and degradation in keratinocytes. 4. Regulation and survival of melanocytes after differentiation. Failure at any point results in either hypo- or hyperpigmentation.

1. Mutations in the gene encoding c-kit, MITF, Pax3, Sox 10, Endothelin 3 and the Endothelin 3 receptor prevent uniform establishment of melanocytes in the epidermis resulting in piebaldism and Waardenburg syndrome types 1-4. Waardenburg manifests with additional symptoms to the hypopigmentation, including heterochromia irides, sensori-neural hearing loss and aganglionic megacolon. 2. Insufficient melanin production in mature melanocytes is due either to failure of protein trafficking to melanosomes, as in Hermansky-Pudlak and Chediak-Higashi Syndromes, or due to mutations that render proteins non-functional, as in oculocutaneous albinism (OCA) types 1-4. The four forms of OCA all result in dysfunctional, either directly due to mutations in the tyrosinase gene (OCA1) or by disruption of protein folding and processing (OCA 2-4). 3. Failure to transfer melanosomes to the keratinocytes occurs due to an inability to transport and maintain melanosomes at the tips (Griscelli syndrome, mutations in Rab27, myosin Va). 4. Once a functional melanocyte has been established in the epidermis, cytokines are required to maintain these cells, which are slow-cycling and not readily replaced. Death of mature melanocytes results in depigmented patches

characteristic of vitiligo. There has been much debate as to the precise etiology of this condition, which has both a genetic and autoimmune component.

Hyperpigmentary disorders can be similarly categorized. 1. Developmental hamartomas are thought to evolve from migrating melanocytes during embryogenesis. They present as at birth as congenital nevi, including nevus of Ota and nevus of Ito. Nevus of Ota and Ito are more common among Asians and females. 2. An epidermal inflammatory response can stimulate an increase in melanocyte activity and subsequent hyperpigmentation. This response is mediated by prostaglandins and leukotrienes. Post-inflammatory hyperpigmentation is also present in patients with Incontinentia pigmenti, which is due to mutations in NEMO/IKKγ, a protein vital for NFκβ signaling. Increased pigmentation is due to the presence of melanophage. 3. Blockage of melanosome transfer can also result in hyperpigmentation as in Peutz-Jeghers syndrome, which results from mutations in the LKB gene that encodes a serine/threonine protein kinase. 4. Increased melanocyte numbers may also account for acquired hypermelanotic conditions such as lentigo senilis, which is common in photoaged skin and is accompanied by increased expression of Endothelin-1, the receptor ET-B-R and stem cell factor. Finally, a number of drug-induced cases of hyperpigmentation have been reported, including in response to bleomycin and fluorouracil. Epidermal hypomelanosis can be treated successfully with laser ablation; however this treatment modality, particularly in the case of congenital melanocytic nevi remains controversial because of the potential for malignancy.

Plenary Symposium 10 - "Pigmentary Disorders #2, Disorders of Hyperpigmentation (Congenital and Acquired Hypermelanoses"

by Marjan Huizing

Dr. Genji Imokawa showed that skin hyperpigmentation after UVB radiation and hyperpigmentation occurring in Lentigo Senilis (LS) occurs via different autocrine mechanisms, involving differential gene expression of the endothelin-1 (ET-1)/endothelin B receptor (ET_BR) as well as stem cell factor (SCF)/SCF-receptor c-KIT cascades.

Dr. Masako Mizoguchi reported that the onset of the pigmented macules in ASDM (acquired symmetrical dermal melanocytosis) is caused by activation of pre-existing, immature melanocytes in these macules. This activation can occur through sunlight, estrogen and/or progesterone, inflammation, a hereditary deposition, surface interaction with elastin, or other unknown factors.

Dr. Hirofumi Aoki presented evidence that locally upregulated interferon-stimulated genes might induce migration of inflammatory cells to delayed pigmented lesions on dorsal skin. Factors excreted by inflammatory cells (such as SCF) can locally induce melanocyte proliferation and melanin synthesis. Anti-inflammatory drugs demonstrated suppression of pigment formation.

Dr. Michihiro Kono described that mutated *ADAR1* (RNA specific adenosine deaminase gene) cause the autosomal dominant disorder Dyschromatosis Symmetrica Hereditaria (DSH). Patients with Dyschromatosis Universalis Hereditaria (DUH) or Acropigmentatio Reticularis (AR), both similar to DSH, did not carry any *ADAR1* mutations, indicating that DUH and AR are different genetic entities.

Roman Garcia created a conditionally over-expressing *Edn3* (Endothelin 3) mouse model, in which pigmentation was studied during embryogenesis and neonatal development with or without administration of oxycycline. Edn3 appeared to induce skin pigmentation.

Dr. Ganesh Diwakar showed increased expression of P-Erk (enhanced MAPK activation) and pigmentation genes (*Tyr*, *Tyrp1*, *Dct*, *Mitf*) in neurofibromin Nf1+/- mouse melanocytes. These effects were reversible by the Mek inhibitor PD98059. It is still unclear if haploinsufficiency of *Nf1* affects

melanocyte development and differentiation through direct effects of Ras activation or through other cellular mechanisms.

Concurrent Symposium 10 - "Photobiology, Photoprotection/Photocarcinogenesis)

by G Ghanem

In this session, two plenary lectures and four talks have been presented.
Merlino *et al*. showed a human melanoma model with deregulated c-met pathway. This model transposed to mice with an inactive INK4a/ARF was susceptible to UVB but not UVA to develop melanoma. The aim of this work was to design a mouse model that can be extrapolated to human for further prevention strategy evaluation.

Young AR developed arguments based on in vivo data suggesting that individual susceptibility to DNA photodamage might be depending on other factors than on solely skin phototype.

Al Kaderaro *et al*. observed that UV induced damage of melanocytes bearing a loss-of-function MC1R is higher than in the one with a functional receptor. This prospective effect on DNA was significant in the presence of α-MSH. The authors also observed a reduction in H_2O_2. Interestingly, they showed that functional MC1R is important to obtain a series of events including MITF phosphorylation, Heme Oxygenase-1 and P53 activation. They concluded that a non functional MC1R may increase the risk for a malignant transformation.

Hauser *et al*., from the same group as above, examined the rate of DNA repair in normal melanocytes with or without impaired MC1R function, after UV irradiation. The authors found a correlation between pigment content and CPD induction, H_2O_2 release and 8-O-dG formation; indicating a protective role in these cells but not in hNM with non functional MC1R. These data support and complement the above stated work from the same team.

Noonan *et al*. presented a model for a UV induced melanoma in HGF/SF transgenic mice, similar to that occurring in man. They crossed these mice on C57BL/6 background with similar animals with a non functional MC1R (recessive yellow). By comparing wild type to transgenic mice after UVA, UVB and visible irradiation, the concluded that pheomelanin might not be involved in melanoma genesis but rather in an aberrant signaling through MC1R.

Steinberg *et al*. described a novel mitochondrial deletion after a single UVB irradiation in an epithelial human cell line. The same could also be observed in different cell types as well. The authors suggested and showed evidence supporting the mechanism of such a deletion.

Concurrent Symposium 11 – Extracutaneous Pigment, Neuromelanin (Oculocutaneous albinism, Parkinson's disease)

by T Sarna

The symposium consisted of two plenary lectures and four oral presentations selected from submitted abstracts. It was co-chaired by D-N. Hu, M. Naoi and T. Sarna. Considering the scope of all presentations, the symposium was rather heterogeneous in nature touching on several different aspects of pigment research. The symposium was well attended and had lively discussion.

New methods for isolation and cultivation of conjunctival melanocytes, obtained from human donor eyes, were described by D-N. Hu, who also discussed differences in the cell morphology and the ability to form melanin *in vitro*, when compared with uveal melanocytes. The fact that conjunctival melanocytes are more similar to epidermal melanocytes is consistent with recent epidemiological

studies, which indicate a significant increase in the annual incidence of conjunctival melanoma, coinciding with the trends seen in cutaneous melanoma. Haugarvoll et al. presented a paper, in which melanogenesis and melanosme transport and secretion was studied in a CD83 teleost leukocytes. It appears that these melanomacrophages, that are thought to participate in immune reactions and free radical trapping, represent a distinct melanin-producing DC population consisting of phylogenetically relict multifunctional cells. Anderson et al. discussed the pigment dispersion syndrome (PD), which is characterized by aberrant deposition of liberated iris pigment and often progresses to elevated intraocular pressure and pigmentary glaucoma. The authors utilized the DBA/2J mouse model to gain insight into the mechanism of the diseases. Results of the study strengthen the hypothesis that aberrant melanosomal processes contribute to the susceptibility of iris toward PD. The role of neuromelanin and neuromelanin precursors, particularly dopaminequinone, in initiating nigral neurons death cascade through oxidative stress, mitochondrial dysfunction and reduction in the activity of ubiquitine-proteosome system was discussed by M. Naoi.

The first plenary lecture, delivered by L. Zecca, was one of the Symposium highlights. The author characterized physicochemical properties of neuromelanin relevant for its postulated protective role in the *substantia nigra* and *locus coeruleus*, stressing the ability of neuromelanin to sequester redox-active metal ions. Although the synthesis of neuromelanin is a protective process since it removes potentially damaging excess of cytosolic catechols and decreases the level of free iron, neuromelanin can also be responsible for neurotoxicity. This could happen if extraneuronal neuromelanin, released by the dying neurons, activates microglia, which release several toxic factors and increase oxidative stress. As a result, a vicious cycle of chronic neurodegeneration occurs.

In the second plenary lecture, M.V. Schiaffino et al. discussed results of their study, which indicated that OA1, a glycoprotein, encoded by the gene responsible for ocular albinism 1, was a resident intracellular protein, containing multiple melanosomal/lysosomal sorting signals, and functioned as a canonical G protein-receptor, capable of activating heterotrimeric G proteins and the associated signaling cascade.

Concurrent Session 12 – Developmental biology

by Lluís Montoliu

The session devoted to Developmental Biology was most interesting and gathered nearly all developmental biologists attending the IPCC meeting, either as speakers or among the audience. Itm included a few last minute changes, according to the original program, which are detailed below in this report. The session was chaired by L. Montoliu and E.K. Nishimura.

The first speaker was Bernier Wehrle-Haller (University of Geneva, Switzerland) who reported the last findings of his group on the relationship between the Kit-ligand dimerization, and its ER-export determinants, that appeared to be controlled by its transmembrane domains. The interaction of the Kitl protein with itself was assessed using elegant cute-edge fluorescence complementation assays. His results suggested that the transmembrane induced dimerization of Kitl in the ER was required for the efficient recognition and cell surface transport of Kitl dimmers by the ER export machinery. Next, Deborah Lang (University of Chicago, IL) presented the work of her laboratory about the role of Pax3 as a regulator for the differentiation of adult melanocyte stem cells. Her results indicated a dual role of this transcription factor, which initially triggers the melanogenesis cascade (by activating the expression of downstream Mitf) and, at the same time, prevents the expression of the Dct gene by covering the Mitf site found at the Dct promoter. This occupancy of the Mitf binding site by Pax3 is not released until Pax3 is removed by activated beta-catenin, thus maintaining an undifferentiated state and leaving the cell poised to differentiate in response to external stimuli. The third speaker was Aaron Thomas (University of California, Davis) who discussed the role of Mitf in the specification of avian melanoblasts. By decreasing the presence of Mitf by morpholinos they could show that Mitf is required to the cell-fate determination of migrating neural crests in their route to melanoblasts. Raising Mitf expression using recombinant plasmids was enough to differentiate neural crest cells to melanoblasts, in vitro. FoxD3 appears to control Mitf expression. Next, Robert Kelsh (University of Bath, UK) illustrated his most recent

findings in the genetic regulation of iridophore development exploring new interactions between *anaplastic lymphoma kinase* (*alk*), a critical compound for iridophore fate specification in zebrafish. Another mutant, *parade* (*pde*) uniquely displays ectopic melanophores and iridophores. Unexpectedly, a double mutant *alk-pde*, having iridophores, suggested an *alk*-independent mechanism of generating this type of pigment cells. The fifth speaker was Robert Cornell (University of Iowa) who presented his work on a cation channel, *trpm7*, that appears to be needed for both differentiation and survival of embryonic melanophores in zebrafish and it is also being found and expressed in human melanoma cell lines. The *trpm7* gene was identified as the cause of the *touchtone* mutation, in which all embryonic melanophores appeared pale, non-dendritic and prone to death. Other members of the TRPM family have a differential lower expression in metastatic melanoma cells, as compared to melanocytes, but this does not appear to be the case with *trpm7*. His results revealed a pathway critical for cell survival of melanocytes and possibly melanoma cells. Next, Stefano Bertuzzi (NINDS-NIH, Bethesda, MD), from Heinz Arnheiter's lab, presented his results on the role of Mitf and Vax transcription factors in retinal pigment epithelium (RPE) specification and development. *Mitf* expression initiates dorsally at RPE whereas *Vax* expression determines the ventral aspect, with both functioning as mutual repressors of each other's expression. The seventh speaker was Thomas Hornyak (NCI-NIH, Bethesda, MD) presented the *microphthalmia-white* mouse mutant model (*Mitf* $^{Mi\text{-}wh}$/+) as an experimental model for human Waardenburg and Tietz syndromes, regarding its auditory defects. His experiments concluded that the hearing defect observed in these mice was associated with strial degeneration and lack of strial melanocytes in the early post-natal period. The observed rescue of otic melanocytes from these mutant mice with epidermal and dermal growth factors indicated how environmental factors may facilitate the selective survival of melanocytes in the hair follicles of these mutant mice. Finally, the session was concluded by a talk presented by Maria Wei (University of California, San Francisco) where another two mouse mutants (*pale ear* and *light ear*), the experimental models for Hermansky-Pudlak Syndromes 1 and 4, respectively, were used to demonstrate that their corresponding gene products do not only regulate melanosomal biogenesis, but also play a developmental role in interfollicular melanocyte function.

Sunrise Session 4 – "Malignant Transformation"

by Nelleke Gruis

The sunrise session on malignant transformation aimed to provide an up-to-date view on the understanding of the molecular genetics of melanoma development as well as transcriptional and signaling mechanisms which underlie the malignant phenotype. In the first overview, provided by Dr. Nelleke Gruis from the Department of Dermatology at Leiden University, Netherlands, discrimination was made between genes and loci currently known to be involved in melanoma predisposition and progression. CDKN2A turned out to be the major melanoma susceptibility gene so far. In melanoma progression, oncogenes and tumor suppressor genes involved in three main cellular pathways: cell cycle regulation, DNA repair and receptor-mediated signal transduction play a role, and provide possible targets for therapeutic intervention.

The second presentation was by Dr. David Fisher from the Melanoma Program at Department of Pediatric Oncology at Dana-Farber Cancer Institute, Harvard Medical School. This talk focused on the MITF transcription factor which plays an essential role in melanocyte lineage development. MITF has been found to transcriptionally regulate expression of numerous components of the pigmentation/differentiation pathway in melanocytes. However, since its mutation affects melanocyte viability (rather than only pigmentation), studies have also focused on its potential role in regulating proliferation or survival. In addition to several transcriptional targets of MITF which play such proliferation/survival roles, the MITF gene itself was recently found to undergo genomic amplifications in 10-20% of primary melanoma specimens. These presentations thus provided an overview of molecular genetic as well as signaling/transcriptional pathways to melanoma tumorigenesis.

Plenary Symposium 11 – "Melanoma 1, Senescence, Immortalization and Progression (Nevi, Melanoma)"

by A Bosserhoff

Plenary Lecture by Meenhard Herlyn "Biology of Melanoma Progression"
Meenhard Herlyn focused on the progression model of melanoma describing the development from nevus to primary melanoma without competence to metastasize to vertical growth phase primary melanoma with competence for metastasis and, finally, to metastatic melanoma. Due to several new findings of his group and in the field this model may have to be adjusted. Melanocyte stem cells have been found in the hair follicle and it is no longer definite that melanoma originates from melanocytes or from melanocytic stem cells. Additionally, recent research led to the characterization of melanoma stem cells. These show high plasticity and can differentiate into melanocytic but also into mesenchymal cell types like fat cells or chondrocytes. These are also the cells in the tumor with high self-renewal capacity. Last but not least, the role of stromal cells should not be underestimated in melanoma development as they can play determining roles for progression and disease outcome.

Plenary Lecture by Estella Medrano "Cellular senescence and Chromatin Remodelers: Possible Mechanism-based Therapeutics for malignant melanoma"
Irreversible growth arrest in cellular senescence is thought to be a potent tumor suppressive mechanism. Estella Medrano could demonstrate this mechanism in primary melanocytes showing growth arrest due to diverse stress, however the cells stay viable over a long period of time. This replicative senescence is controlled by the RB/p16/HDAC1 pathway whereas p53 is not involved in this process in melanocytes. Marker of this process are e.g. upregulation of expression of SPARC and downregulation of expression of MITF or cyclin E. Deregulation of p16 and/or RB is commonly found in melanoma possibly resulting in deregulation of induction of senescence. Additionally, Medrano presented that a critical balance of HDAC1 as chromatin remodeler is required maintaining cellular homeostasis and proliferation in normal cells, which is dysregulated in cancer cells.

"Senescence of human melanoma cells following activation of PKC and MAPK pathways" by PG Parsons
Parson et al. presented that treatment of B16 melanoma with PKC-activating ingenol ester PEP005 resulted in cure of mice. Treatment of human melanoma cell lines with TPA led to terminal growth arrest of sensitive cells after 24 hours with loss of telomerase activity whereas insensitive cells were resistant. Use of a PKC inhibitor (bisindolylmaleimide-1) confirmed the role PKC in this process. Microarray studies demonstrated the repression of E2F1 and E2F1-sensitive genes in the sensitive cell lines. PKC activation in the sensitive cells also resulted in activation of ERK1/2 which was required for growth arrest and cell cycle block. The resistance in several cell lines was possibly due to expression of H-rev107, an inhibitor of the MAPK pathway. The group suggested that PKC activation may play a role in the natural regression of melanocytic lesions.

"The cleavage of MITF by caspases plays an essential role in melanocytes and melanoma cell apoptosis" by L Larribere
Larribere et al. identified MITF (microphthalmia associated transcription factor) as a new substrate of caspases during apoptosis. MITF is known to play a key role in melanocyte development, survival and differentiation. Furthermore, an impact of MITF on melanoma development is speculated. It was presented that MITF processing by caspases mediates melanoma cell apoptosis. A non-cleavable form of MITF renders melanoma cells resistant to apoptotic stimuli. Additionally, the c-terminal fragment generated by caspase cleavage promotes caspase 3 activation, morphologic changes and increases in the sub-G1 population. This finding again points to the functional duality of MITF in survival and cell death that was postulated in several talks on this IPCC meeting.

"Stable overexpression of Smad7 in human melanoma cells inhibits their tumorigenicity in vitro and in vivo" by A Mauviel
Mauviel et al. investigated the effect of Smad 7 overexpression on melanoma cell properties to analyze whether autocrine effects of TGFß are essential for malignant melanoma cells. Smad 7 is known to

inhibit Smad phosphorylation at the TGFß receptor. Smad 7 overexpression did not change the proliferative potential of cells. However, the capacity to invade Matrigel was strongly reduced, likely by downregulation of MMP-2 and MMP-9 secretion. Additionally, anchorage independent growth and tumor formation in nude mice was reduced. The data suggest that TGFß affects the tumor microenvironment as well as the tumor cells themselves by contributing to tumor aggressiveness.

"Further development of human skin xenografts towards modeling melanoma" by A Yoneta
Yoneta et al. present the development of a new skin graft model using skin reconstructs which reduce the problem of donor-dependent heterogeneity. Additionally, lentiviral vectors were applied, which result in stable expression of the transduced transgene. Using this model, melanocytes, keratinocytes and/or fibroblasts can now be stably transduced and then incorporated into the skin reconstruct. It was already demonstrated using this new model system that melanocyte proliferation is induced after transduction with ET-3 or bFGF.

Plenary Symposium 12 – "The Malignant Phenotype"

by Dorthy Bennett and Zalfa Abdel-Malek

In his keynote presentation, Jonathan Rees gave his perspective on pigmentary and non-pigmentary factors that influence the responses of skin to ultraviolet radiation (UV). The role of pigment in the UV response is well-established, however, other factors need to be considered, such as the *MC1R* genotype, which Rees and his co-workers found to be associated with freckling and hair color, but not necessarily with skin color, and to have a dosage effect. Expression of the wild type *MC1R* was associated with the highest ratio, while heterozygosity and homozygosity for alleles that reduce the function of the MC1R were associated with an intermediate and the lowest ratios of eumelanin to pheomelanin, respectively. Recently, Rees reported that eumelanin to pheomelanin ratios do not differ markedly in the skin of Asians vs. North Europeans, and that both eumelanin and pheomelanin were increased following UV exposure. These data refute a specific role for pheomelanin in the damaging effects of UV, and the usefulness of pheomelanin content alone, or eumelanin to pheomelanin ratio, in predicting the response to UV. One additional factor that needs to be considered is epidermal thickening, which confers photoprotection and is more pronounced in Northern European than Asian skin.

Dorothy Bennett gave the IFPCS Presidential lecture entitled "the genetics of melanoma". She reviewed evidence that melanoma development requires fewer events than for other solid tumors. Germline susceptibility to melanoma is partly determined by the *CDKN2A* locus, encoding two tumor suppressors, p16 and ARF. The main function of p16 is as an effector of senescence, particularly M0. p16/RB defects lead to M0 deficiency but then M1 senescence which is p53-dependent and associated with short telomeres. Additional blockage of p53 extends lifespan further, and leads to M2 (crisis), when cells divide and die. p16-deficient cultured human melanocytes exhibit delayed, p53-dependent senescence, and high apoptotic rate that can be inhibited by keratinocyte-derived survival factors. This raised the question: "are moles the result of senescence in the skin?" A model for melanoma progression was presented. In the model, nevi arise through an activating mutation in *BRAF* or *NRAS*, leading to M0 senescence. Defects in p16 would lead to dysplastic nevi, M1 senescence and M2 crisis, then activation of hTERT leading to immortal RGP melanoma. Further mutations allow progression to invasive VGP melanoma. On testing the model, nevi did show markers of senescence, especially inability (6/6) to proliferate in culture. Phospho-CHK2 and phospho-p53^{ser20}, part of the ATM-mediated DNA damage response, are possible markers of M1 and M2. Both were observed in most benign lesions but expression increased with progression. hTERT expression also increased with progression and was highest in VGP melanoma, while p16 was lost.

Robert Weinberg provided a stimulating and fast-moving talk, in the area of genetic changes required for the development of cancer. His group has published sets of genetic changes that can produce

cancers from various normal mammalian cell types. Here he presented recent findings on human breast cancer and melanoma. He discussed changes found in breast cancer stromal cells (fibroblasts), as opposed to the cancer cells. When purified, these fibroblasts were abnormally able to stimulate angiogenesis and attract endothelial precursor cells, indicating either genetic or epigenetic changes in these cells, or that stromal cells could arise from tumor cells. He also discussed microarray analyses of genes overexpressed in metastatic cancer cells. In breast cancer these included TWIST, which is involved in normal epithelial-mesenchymal transitions in development. Genes apparently overexpressed in metastatic melanoma included Slug (SNAI2) and FOXC2, again developmental effectors. Interactions could be found; for example exogenous TWIST expression could upregulate SNAI2. Some of these findings appear in Nature Genetics, October 2005.

Plenary Symposium 13 – "Melanoma 2 Genetics, Susceptibility, Epideniology, Etiology and Therapy (Melanoma)"

by Frank Meyskens

The general theme of this session encompassed the genetics, susceptibility, epidemiology, etiology and treatment, with a predominant emphasis on the human malignancy. Two plenary lectures (Chin, Marais) and four competitive abstracts comprised this session.

Lynda Chin (Harvard) covered two major areas. She described a new transgenic model that involves an inducible ras in the setting of Ink4a/Arf^{-} which resulted in an amelanotic melanoma phenotype. The majority of her talk was spent on describing a complex gene discovery program defined by programmable array-CGH. Many new potential targets of unknown function were identified.

Roge Marais (London) discussed the b-Raf system in detail including the various relationships between the ras-raf systems and among the a, b and c forms. Of considerable parenthetic interest was his description of b-Raf mutations in murine bronchiolalveolar lung lesions.

Frank Meyskens (UC Irvine) updated the evolving story on redox and the pathogenesis of melanoma. Abnormalities in redox regulation as related to NADPHoxidase, melanin conversion from an antioxidant to pro-oxidant, melanosomal disruption and mitochondrial mutations all seem to contribute to high levels of reactive oxygen species (ROS) in melanoma. A number of new preventive and treatment strategies were described based on this paradigm.

Sun Yang (also of UC Irvine) continued these themes and described the adaptive response to elevated levels of ROS which occurs including activation of many transcription factors and of the multifunctional protein apurinic/apyrinidic, endonuclease/redox effector factor-1(APE/Ref-1) which both assists in DNA repair as well as modulates the redox state of many transcription factors. Initial studies of an inhibitor of APE/Ref-1 were described with the polyphenolic antioxidant resveratrol being a lead compound.

Yutaka Kawakami (Tokyo) described the development of individualized immunotherapy by identification of highly immunogenic antigens using rapid serum IgG antibody screening or in vitro T-cell induction. Following this screen injection of dendritic cells based on reactivity to the individual immunogenic antigens produced induction of $CD8^{+}$ CTL and regression of large untreated tumor located at a remote site. Based on these encouraging murine studies, a clinical phase I/II trial has commenced.

Finally, E Hacker (Australia) using the $CDK4^{R24C/R24C}$/TPRAS mouse system demonstrated that a single dose of ultraviolet light radiation(UVR)induced metastatic melanoma in neonatal mice but not in adult animals, even when multiple doses of UVR were administered. Detailed studies suggested

that Ras activation was sufficient to predispose melanocytes to UVR-induced transformation but mutant Cdk4 was necessary for progression to large and/or aggressive metastatic lesions.

Overall these six presentations from four countries indicate that studies of human melanoma are alive and well and that a wide range of approaches are being used to offer better platforms for understanding the etiology and pathogenesis of the disease as well as designing new preventive and therapeutic treatments.

Concurrent Session 13 – "Melanoma Basic Research"

by Faith Strickland

Basic research conducted to understand the processes involved in transformation of melanocytes to malignant melanoma focuses on the steps involved in tumorigenesis: initiation, promotion and progression. The role of nuclear receptors in melanoma carcinogenesis was explored by Indra et al. The chemopreventive action of retinoids is mediated by heterodimerization between retinoid X receptor (RXR αβγ) and other receptors such as the vitamin D receptor (VDR) and peroxisome proliferators activated receptors (PPARS). Selective ablation of RXRα and PPARS in epidermal keratinocytes greatly enhanced nevi formation as well as increasing the numbers of papillomas and progression of papillomas to carcinomas in the two step chemical carcinogenesis protocol of DMBA initiation and TPA promotion. Nevi progressed to melanomas only in the RXRα-null animals and unlike the papillomas, malignant transformation of melanocytes was not blocked by treatment with glucocorticoids. Their data suggested that a paracrine mechanism is involved in the formation of melanomas and that distinct mechanisms are involved in the formation of melanomas compared with carcinomas in their model. The BRAF-cyclin D1 pathway that controls $p27^{Kip1}$ via Skp2 (an F-box protein) may contribute to deregulation of cell cycle progression in melanoma cells. BRAF regulates cell cycle control, Skp2 and MAPK activation. Mutations in BRAF led to increased Skp2, activation of MAPK signaling and subsequent proliferation (Bhatt et al.). Mutations in BRAF are found in many melanomas, however, Namkoong et al. reported that MAPK could also be activated in cells with wild type BRAF through overexpression of the metabotropic glutamate receptor 1 (Grm1). Nevertheless, overexpression of Grm1 alone is insufficient to bring about a fully transformed phenotype in melanocytes and requires additional growth factors to express continuous growth and anchorage independence (Shin et al.). Once cellular transformation and escape from growth control occurs, progression to metastatic disease requires further changes such as anchorage independence, expression of proteases to enhance migration, and freedom from growth control by the local tissue environment. The human *HUGl-1* gene which has significant homology to the *Drosophila* tumor suppressor gene *lethal (2)giant larval* was found to be reduced or lost in melanoma cell lines and tumors and the loss was associated with the advancement of the disease stage (Bosserhoff et al.). *HUG-1* expression downregulated MMP2 and MMP14 and increased E-cadherin thus, controlling melanocyte adhesion and migration. Beermann et al. used a transgenic mouse line expressing the melanocyte-targeted dominant activated human $N\text{-}Ras^{Q61K}$ in combination with an absence of $p16^{INK4a}/19^{ARF}$ to show that these mutations were sufficient to cause metastatic melanomas in 90% of the animals. Addition of FGF (bFGF, FGF2) had no effect on melanoma genesis, indicating that under conditions of major pathway dysregulation, additional growth factors may not be needed to induce melanoma progression. Proteins controlling cellular proliferation which are dysregulated in melanoma can be used as both markers for disease and potential therapy. Matsuzaki et al. reported a newly discovered protein FABP7 that controls proliferation and migration of melanoma cells in vitro and is expressed in high levels in both cell lines and melanomas isolated from tissues. Over half the patients with melanoma in their study also had serum antibody to FABP7 making this protein a potential marker for the disease. Finally, Miao et al reported creating a new class of metal cyclized melanotropin peptide analogues (CCMSH) that bind the melanocortin-1 receptor. These compounds can be used as radiopharmaceuticals for melanoma imaging and therapy. Taken together, the data presented at this

session helped to shed new light on the complex interaction of pathways involved in regulation of cell growth and their role in melanoma development.

Concurrent Session 14 – "Hypo- and Hyper Pigmentation"

by Jim Nordlund

BK Goh talked on the loss of melanosome transfer accounts for guttate leukoderma in Darier's disease. Darier's disease is a genetic disorder in which the face, neck and trunk are covered with gritty, keratotic papules. There are mutations in a gene ATP2A2 that alters intracellular calcium homeostasis. In type 3 Asian skin guttate hypopigmented macules are observed. Electron microscopic studies from these macules demonstrate melanosomes are present in the dendrites but are not transferred to surrounding keratinocytes. The findings are consistent with dysfunction of calcium metabolism. It is not clear why these white macules are observed only in Asians with Darier's disease.

Hermansky Pudlak syndrome is caused by mutations in at least 7 different genes in humans. Protein products from normal genes form complexes (Westbroek et al.). Attempts to study binding of these proteins in fungi was not successful. Studies on proteins from normal human cells did show HPS proteins 5-3-6 form a complex. Such results permit additional investigations on complexing and binding of the various 7 HPS proteins.

Various species of chickens are hatched with pigmented feathers but lose color after molting. In the barred rooster, it is thought that toxic substances cause the barred appearance, possibly oxygen radicals. Glutathione was deficient in cells from the barred chicks. Melanocytes in cultured were rescued by addition of catalase and glutathione, an observation that is consistent with oxygen toxicity as a cause of barring in chicken feathers.

Synergistic therapies for vitiligo was presented by A. Ramaiah. Standard therapies for vitiligo include PUVA, topical steroids and similar agents. bFGF is a known mitogen for melanocytes. A molecule derived from bFGF has been applied to depigmented skin and produced repigmentation in some patients. In combination with PUVA or other standard therapies, repigmentation was observed. The bFGF derivative might be a useful commercial product to treat vitiligo.

Information on a controlled study using Levamisole was presented by M. Ramam. Levamisole, an immune modulating medication, has been proposed as a medication for vitiligo. In a double blinded study, patients with vitiligo received either levamisole or a placebo. There was no observable difference between the treated and placebo group. Levamisole may not be effective for treating vitiligo.

Vitiligo can be treated with surgical transplants. Mac Neil et al. have developed a complex, expensive system for growing and amplifying keratinocytes and melanocytes into sheets that are life saving for treating patients with burns. The technique has potential for treating patients with vitiligo.

Repigmentation of patients with vitiligo is thought to depend on stem cell melanocytes in the niche are of hair follicles. L.M. Davids presented preliminary data to confirm this idea. However no studies have shown proliferation of melanoblasts in the niche area following treatment of PUVA. Results of this study have confirmed that single hair follicles can be obtained and maintained in culture. So far it has not been possible to demonstrate melanocyte proliferation in the niche area. These techniques will allow documentation of melanoblast behavior within the niche area following treatments such as PUVA.

Concurrent Session 15 – "Immunology"

By I Caroline LePoole

The melanocyte stands out for its melanin synthesis and because the organelles supporting this process make for a uniquely immunogenic cell type. Within the immunology session, abstracts were presented on immune responses to melanocytes and melanoma cells.
J van den Boorn presented a characterization of T cells that were derived from actively depigmenting skin biopsies of 7 vitiligo patients. Vitiligo T cells were found to be reactive mostly with MART-1 (up to 35% of isolated T cells) and tyrosinase, supporting intriguing similarities among vitiligo and melanoma. J Pawelek discussed the presence of LPHA expressing Langerhans cells (LC) in vitiligo epidermis, indicating that LC activation is apparent in actively depigmenting skin. IC Le Poole demonstrated that regulatory T cells were virtually absent from actively depigmenting vitiligo skin, contributing to the perpetuation of an immune response. LM Hopkins showed that several phosphorylated peptides are unique to cancer cells, describing some 10 candidate phophopeptides that may become part of future anti-tumor vaccines.

Concurrent Session 16 – "Extracuetneous Pigmentation

by William Oetting

Baranova spoke on melanin synthesis in fat tissue from morbidly obese individuals. It was shown that expression of melanogenic proteins is upregulated in adipose tissue in morbidly obese individuals. Mason-Fontana staining of tissues confirmed the presence of melanin biosynthesis in these tissues. E. Greggio talked on Parkinson's disease (PD). PD is characterized by the progressive loss of dopaminergic neurons of the substantia nigra pars compacta. They collected evidence of tyrosinase expression in the brain. There was a question if variation in tyrosinase activity was associated with variations in PD. They report that haplotype analysis of the tyrosinase gene did not exhibit any genetic association between tyrosinase activity and PD. A Gallone looked at melanin biosynthetic activity in the liver. Tyrosinase was shown to be present in Rana Esculenta L liver. The melanogenic system of the liver was further discussed. Using ultraviolet free electron laser photoelectron emission microscopy (UV-FEL-PEEM), WD Bush analyzed the oxidation potential of melanin in the Substantia nigra region of the human brain was analyzed. Analysis was also done with atomic fource microscopy and scanning electron microscopy.

Report on the 19th IPCC Satellite Symposium on Vitiligo
23 Sept 2005, Reston, Virginia

This one-day symposium was sponsored by the International Federation of Pigment Cell Societies (IFPCS), and organised by Alain Taïeb and Mauro Picardo who are currently chairing the special interest group on Vitiligo of the IFPCS. Vince Hearing, the organizer of the 19th IPCC, was very kind to help the logistics of this satellite meeting.
The main objective of the meeting was to foster cooperation within the international community of clinicians and scientists present at the meeting around this common but poorly understood disease that causes much distress especially in dark-skinned communities. Patients' support organisations from all over the world had also been invited to attend and to speak. Maxine Whitton, from the UK vitiligo Society, who could not attend, sent a very thought-provoking paper to the organizers, which helped to shape the discussion on topics ranging from classification of vitiligo and its possible implications for treatment, nature of depigmentation, epidemiology, sunlight and UVB, to basic science and treatment.
The symposium programme took into consideration the IPCC main programme which had already covered a broad range of topics, with two plenary lectures, Raymond Boissy on contact depigmentation and Richard Spritz on the genetics of non-segmental vitiligo, as well as several oral communications and posters (**see list in Appendix 1**). It was decided to give some extra time for the discussion of posters from the main meeting corresponding to the focuses of the satellite sessions, after a summary presentation by the authors.

Session I: Definition of disease, assessment and outcome measures
Chairs: M Picardo and A Taïeb

1. Alain Taieb[1] (Bordeaux, France) presented the work done on this topic by the Vitiligo European Task force (VETF). Alain Taieb mentioned that he wanted to build on his previous fruitful collaborative work for atopic dermatitis, which, through the European Task Force on Atopic Dermatitis (ETFAD), has produced over the last 15 years a widely accepted scoring system (SCORAD), a standardisation of atopy patch tests, and position papers on this disease. His presentation gave a special emphasis on the assessment workshop held in Rome on January 16, 2005 (**IPCC Poster 051, PCR 18, suppl 1, p 68-9**). The VETF was created in 2003 during the ESPCR in Ghent organized by Jean Marie Naeyaert (**Fig 1**) to design tools for clinical research in vitiligo and promote collaborative studies. Two subsequent workshops were held in Paris in 2004 with the aim to design a common evaluation/scoring form which was tested at 11 European clinical centres on 180 patients included in a common database managed in Rome by the San Gallicano Group's statistican, Mario Pellicciotta.

Fig 1

[1] On behalf of VETF members: A Alomar (Barcelona), D Bennett (London), M Böhm (Münster), Y Gauthier (Bordeaux), D Gawkrodger (Sheffield), S Moretti (Florence), T Passeron (Nice), G Leone and M Picardo (Rome), M Olsson (Uppsala), G Orecchia (Pavia), K Ongenae, N van Geel, JM Naeyaert (Ghent), W Westerhof and JP Wietze van der Veen (Amsterdam),

The VETF has chosen to use the simplified **classification of vitiligo**, following Koga's 1977 paper distinguishing segmental and non-segmental forms of disease, but based more on pragmatic than on pathophysiological grounds. However the VETF data collected allows a more accurate classification if needed (including focal, mucosal, acrofacial, common generalised, universal and even more subgroups) since the topography of lesions is reported in the assessment form. The VETF **consensus definition** for **non-segmental vitiligo** is as follows: an acquired, chronic, pigmentation disorder, characterised by white patches, often symmetrical, usually increasing in size with time, and which are due to a substantial loss of functioning epidermal and /or hair follicle melanocytes. The counterpart of this consensus definition is a list of disorders to exclude, namely piebaldism and other monogenic hypomelanoses, including tuberous sclerosis; post inflammatory depigmentation, including mycosis fungoides; post infectious depigmentation such as that seen in pityriasis versicolor, leprosy; post traumatic leucoderma; melanoma-associated leucoderma; melasma; toxic and drug-induced depigmentation (topical and systemic).

The Rome San Gallicano Dermatological Institute (SGDI) workshop was presented to the audience. Its objective was (1) to further test how practical is the VETF evaluation system (**Appendix 2**) which includes 3 main items related to extent, spreading, and staging, and (2) to assess inter-observer variations in scoring patients. 10 dermatologists from 9 European centres could examine 13 patients (8 NSV, 5 SV). For each patient a patch to be assessed was chosen by the organising SGDI team in order to reduce the duration of the session. The patient's opinion about the progression of the selected patch was recorded separately and did not influence the investigators. The dermatologists did not communicate during the session. 130 evaluations of the 3 scoring items were collected and analysed *vs* standardized colour and black and white UV photographs (prototype instrument, Deka, Florence, Italy). The workshop validated the clinical use of the assessment form proposed and showed an overall good concordance among panelists. It however raised several problems, e.g. staging since colour of the selected patch is not homogeneous especially in segmental vitiligo. Staging chosen by investigators reflected generally the worst stage. This poses problems when a few white hairs are present in association with skin repigmentation. A proposal was made for simplifying the staging system (stage 4 deleted). Another difficulty was related to the need to magnify the lesions to assess hairs, especially vellus hairs. Wood's lamp equipment for vitiligo assessment should include a magnifying lens. Analysis of spreading (progressive/stable/regressive) was the most difficult item in a blind (not patient influenced) test. Surprisingly, the investigator's opinion was right in the majority of cases if the patient's opinion was chosen as the gold standard. Overall, it was felt that this item should be graded more accurately using the patient's opinion.

Alain Taïeb summarized his most important points: (1) The SGDI workshop validated a simple clinical assessment system of vitiligo, which can be easily handled in clinical practice. (2) Proposals for simplification of the tested EVTF assessment system were made for the grading grid. (3) Scorer profiles underscore the need of training to decrease inter-observer variability. He delineated desirable further steps, namely (1) including staging and spreading in the initial assessment of patients, in order to build a global index, most important for therapeutic indications and prognosis, which could be understood as an equivalent of the TNM system for cancer; (2) further large scale tests are needed in clinical trials (to check reproducibility~~,~~ and sensitivity), and refinements using automated devices should be encouraged for special purposes, as well as teaching tools, which could be posted on an internet site, such as the ESPCR website. (3) An international agreement for classifying, staging and scoring vitiligo could be set as a main objective of the IFPCS special interest group on Vitiligo.

2. **Ilt Hamzavi** (Detroit, USA) presented the work he published recently with his colleagues while at the University of British Columbia in Vancouver[2] which uses a quantitative parametric score, named the VASI score for Vitiligo Area Scoring Index, which is conceptually derived from the PASI score

[2] Hamzavi I, Jain H, McLean D, Shapiro J, Zeng H, Lui H. Parametric modeling of narrowband UV-B phototherapy for vitiligo using a novel quantitative tool: the Vitiligo Area Scoring Index. Arch Dermatol. 2004 Jun;140(6):677-83.

widely used for psoriasis. He made the point that many vitiligo treatments have typically been analysed using nominal binary scales in which the proportion of treated patients who either do or do not achieve a specified degree of repigmentation is reported and compared by nonparametric statistical approaches. The degree of repigmentation that defines success has often been set previously somewhat arbitrarily at 50% to 75% repigmentation based largely on the global impression of the overall response. Quantitative methods provide data that are generally more intuitive and meaningful to patients and physicians, while at the same time being more sensitive for detecting significant subtle treatment effects. In addition, a quantitative method for measuring vitiligo severity would allow more studies to be compared across a range of data sets. A simple quantitative technique could standardize vitiligo outcome measurements and allow more studies to be included in meta-analyses.

In VASI, the body is divided into 5 separate and mutually exclusive regions: hands, upper extremities (excluding hands), trunk, lower extremities (excluding the feet), and feet. The axillary and inguinal regions are included with the upper and lower extremities, respectively, while the buttocks are included with the lower extremities. The face and neck areas are not included in the overall evaluation. One hand unit, which encompasses the palm plus the volar surface of all the digits, is approximately 1% of the total body surface area and is used as a guide to estimate the baseline percentage of vitiligo involvement of each body region. Depigmentation within each area was estimated to the nearest of 1 of the following percentages: 0, 10%, 25%, 50%, 75%, 90%, or 100% (**Figure 2)**

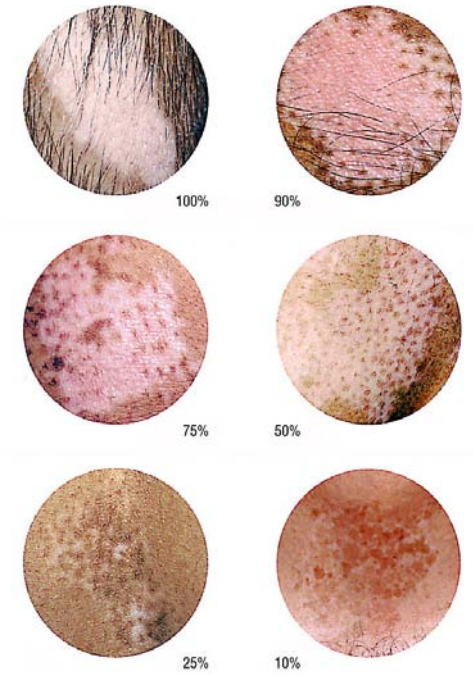


Figure 2: **%** depigmentation visual scale

For each body region, the VASI is determined by the product of the area of vitiligo in hand units (which was set at 1% per unit) and the extent of depigmentation within each hand unit–measured patch (possible values of 0, 10%, 25%, 50%, 75%, 90%, or 100%). The total body VASI is then calculated using the following formula by considering the contributions of all body regions (possible range, 0-100):

VASI = $\Sigma_{\text{(all body sites)}}$ (hand units) x (depigmentation)

Dr Hamzavi stressed that using quantitative scales can more easily capture sequential trends in response by time or treatment number. Although such data for vitiligo are currently unavailable, they are nevertheless important because at the present time patients with vitiligo are asked to commit to treatment for a year or more based largely on knowing only the probability of achieving a certain specific degree of repigmentation at the end of therapy without any actual data on the expected rate of

response over time. The VASI provides a sensitive method for detecting treatment responses, as evidenced by the demonstration of a significant difference between NB–UV-B and control within 2 months of treatment. If the Archives' published study had used a nonparametric method to evaluate response and chosen the usual 75% repigmentation threshold as representing treatment success, the trial would have shown a non-significant result (P = .50 by the McNemar test) instead of the highly significant difference found using the VASI (P<.001). Also, the VASI provides information over a range of time points rather than an arbitrarily set end point. When compared, the correlation with the VASI was lower for patient assessment than for physician assessment but–was still statistically significant. The difference may be owing to a wider variation in what patients perceive to represent improvement. Dr Hamzavi said that it was important for other investigators to evaluate the validity of this technique, and he believes it is a quick and reliable tool, which can be applied to any setting and treatment.

3. Other papers of this session were presented by David Gawkrodger (Sheffield, UK) and A.J. Kanwar (Chandigarh, India).

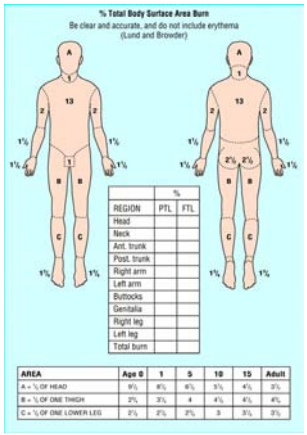
Dr Gawkrodger presented a classification of vitiligo according to clinical pattern and disease association, based on the prospective evaluation of 41 adult patients, stressing the importance of a careful clinical evaluation. He found a 18% family history of vitiligo, 34% of patients had autoimmune thyroid disease, and 33% a family history of endocrine autoimmune disease.

Dr Kanwar examined 212 patients with onset of vitiligo after 50 years of age. Associated endocrine autoimmune disorders were present in 21.4% and 15.9% had a family history of vitiligo. Segmental vitiligo is rare in this age group (5.4%).

Discussion:

Dr M Ramam (New Delhi, India), suggested using the Lund Browder chart to score extent, because its advantage over the "rule of nines" is that the denominator is smaller for many body areas. As pointed out during Dr Taïeb's presentation, the difficulty in assessing surface area involvement is greatest when the denominator is large as on the legs. He further suggested that studies reporting on the response to treatment in vitiligo mention how many patients have achieved complete (100%) repigmentation. He said that this information is usually pooled together with those who have more than 75% repigmentation. However, for patients and those treating vitiligo, the difference between 100% repigmentation and 90% repigmentation is great.

Fig 3: Figure of the Lung and Browder chart reproduced from Hettiaratchy, S. et al. BMJ 2004;329:101-103



Another point was made concerning Dr Hamzavi's presentation by Dr Taïeb, indicating that multiplying extent by lesional score as in PASI is a potential source of error and increased variability between investigators, which has been avoided in other scales such as SCORAD which is an additive index combining extent, intensity and subjective signs.

Among questions taken from **Maxine Whitton's** list:
Prevalence of vitiligo: experts in the audience agreed on a population based prevalence around 1% or less, but this figure might be increased in particular ethnic backgrounds.
Location of disease and resistance to treatment: **Dr Nordlund** (Cincinnati, USA) replied to the question of differential responses to treatment according to location of disease, by emphasizing the role of the reservoir of melanocytes which is found in hair follicles and absent on mucosal and glabrous skin such as the hands, and the role of precipitating/ environmental factors, especially trauma (Koebner's phenomenon).
Segmental and non segmental vitiligo, a different disease or just a type of vitiligo? The hypothesis of a mosaicism for a major predisposing gene especially in stemness genes was already proposed but not yet investigated[3], and there are cases associating NSV and SV which point to applications of the concept of type II mosaicism[4] in multigenic diseases, corresponding in the segmental lesion to a possible double dosing of the major predisposing gene, and a more recalcitrant form of vitiligo in the corresponding area (Dr Nordlund, Dr Taïeb).

Session II: Evidence-based therapy and future trends
Chairs: W Westerhof (Amsterdam, NL), J Nordlund (Cincinnati, USA), Y Gauthier (Bordeaux, France)

There were four invited presentations, made by Dr Davinder Parsad (Chandigarh, India) on medical treatments, Dr Mats Olsson (Uppsala, Sweden), on surgical treatments, Dr Pearl Grimes (Los Angeles, USA), on combined therapies, and Dr H Lim who could not join the meeting was replaced by Dr Hamzavi (Detroit, USA) on phototherapies.

A Cochrane systematic review of interventions for vitiligo is currently in press as mentioned in her presentation sent to the organizers by Maxine Whitton who worked on this review with Dr Urba Gonzales from Barcelona and Darren Ashcroft, statistician from Manchester. This review could assess 19 randomised controlled trials (RCT) with an overall poor methodology, since the method of randomisation was rarely described, and that only 9 studies were double blinded. There was overall weak evidence for the effectiveness of interventions for vitiligo, including alternative and experimental. All measures of outcome related to re-pigmentation, while none considered depigmentation, cosmetic camouflage, or psychological interventions. No pooling was possible since no two trials compared the same intervention. The design was different between studies (left/right comparison, individual patches assessed, or comparisons between groups). Interestingly, placebo effects seemed limited, since in many trials none of the patients who received placebo improved. The relative risks and confidence intervals were large, inducing a high level of uncertainty. Important observations were made in this review: 1. There are large variations in methods for scoring repigmentation 2. There are no reliable data on patient-centred outcomes or quality of life measures. 3. There is a lack of any reliable measure of outcome. 4. Trials are too short to assess effectiveness or adverse effects. 5. Few studies are done in children. 6. Age, duration, type (segmental responds best to surgery), and choice of site (face versus limb extremities) could affect outcome. 7. Lack of consensus about a cause leads to a multiplicity of treatments. 8. No clear clinical guidance emerged from the review, but some implications for research priorities: there is a need for more basic research

[3] Taieb A.Intrinsic and extrinsic pathomechanisms in vitiligo. Pigment Cell Res. 2000;13 Suppl 8:41-7.
[4] Poblete-Gutierrez P, Wiederholt T, Konig A, Jugert FK, Marquardt Y, Rubben A, Merk HF, Happle R, Frank J. Allelic loss underlies type 2 segmental Hailey-Hailey disease, providing molecular confirmation of a novel genetic concept. J Clin Invest. 2004 Nov;114(10):1467-74.

on causes; agreement on classification and standardised measurement of outcome; translational research from scientific discovery to RCT, and more research into psychological interventions. Patient-centred outcomes would improve study designs.

Most of these difficulties outlined in the review were considered in the invited oral presentations given at the meeting. Dr Parsad emphasised also the various effects of treatments on repigmentation patterns and stability [5]. Diffuse repigmentation is the least stable. Psoralens predominantly exhibit a perifollicular pattern of repigmentation and steroids (topical/systemic), a diffuse type. The speed of repigmentation is much faster when initial repigmentation is of the diffuse type as compared with follicular repigmentation. Marginal and perifollicular repigmentation are more stable than the diffuse type of repigmentation. Dr Olsson made remarks on the selection of patients for surgical procedures and specific transplantation methods[6]. Dr Hemzavi indicated that the evidence for effectiveness of UVBTL01 was the best following the pioneering work of Dr Westerhof in Amsterdam, both for adults and children[7]. Targeted phototherapy using excimer 308 xenon-chloride laser or monochromatic excimer light (MEL) are promising for some locations and limited disease. Combination with tacrolimus is synergistic. Dr Grimes emphasised the role of calcineurin inhibitors (tacrolimus, pimecrolimus), which are immunosuppressive, in combination with surgical and phototherapy interventions. A summary of the current approaches to treatment can be found in the following Table.

Type of Vitiligo	Usual management
Segmental (includes focal and mucosal)	**First line**: Avoidance of triggering factors, local therapies (corticosteroids, calcineurin inhibitors). **Second line:** Localized UVB therapy, especially Excimer lamp or laser. **Third line**: Consider surgical techniques if repigmentation cosmetically unsatisfactory.
Non segmental (including acrofacial)	**First line**: Stabilization with UVB TL01 therapy, at least 4 months. Combination with systemic/ topical therapies, including reinforcement with localized UVB therapy, possible. **Second line:** Consider surgical techniques in non responding areas especially with high cosmetic impact. However, Koebner phenomenon limits the persistence of grafts. Relative contraindication in areas such as dorsum of hands.

[5] Parsad D, Pandhi R, Dogra S, Kumar B. Clinical study of repigmentation patterns with different treatment modalities and their correlation with speed and stability of repigmentation in 352 vitiliginous patches. J Am Acad Dermatol. 2004;50:63-7.

[6] Olsson MJ, Juhlin L.Long-term follow-up of leucoderma patients treated with transplants of autologous cultured melanocytes, ultrathin epidermal sheets and basal cell layer suspension. Br J Dermatol. 2002;147:893-904.

[7] Westerhof W, Nieuweboer-Krobotova L. Treatment of vitiligo with UV-B radiation vs topical psoralen plus UV-A. Arch Dermatol. 1997;133:1525-8. ; Njoo MD, Bos JD, Westerhof W.Treatment of generalized vitiligo in children with narrow-band (TL-01) UVB radiation therapy. J Am Acad Dermatol. 2000;42(2 Pt 1):245-53.

TABLE 1. General outline of management for vitiligo (adapted from Taieb, 2005, in press)

Five short presentations were given on tissue culture techniques (S Mac Neil, Sheffield, UK, and DN Hu, New York, USA), physical treatments namely Excimer Laser (A Overbeck, Madrid, Spain) and low energy helium-neon laser (CS Wu, Taipei, Taiwan), and adjuvant growth factor therapy (A. Ramaiah, Hyperabad, India). Dr Mac Neil presented the data (**PP053, OP 120**) which are promising in terms of delivering to distant centres melanocytes grown on a chemically defined surface (acid and amine plasma polymers) which have been transferred successfully to an in vitro model of human dermis. Dr Dan-Ning Hu showed an update of a study using pure autologous melanocytes in 150 vitiligo patients Taiwan[8], with better results in segmental and stable generalised cases. Dr Wu (PP046), working on the assumption that SV results from the dysfunction of sympathetic nerves in the affected areas, expanded his earlier observations[9] in an updated series of 40 patients, indicating that low energy helium-neon laser 632.8nm can repair nerve injury and improve SV. Dr Overbeck shared his experience with the excimer laser and showed encouraging results with a combined blister graft plus excimer laser technique. Dr Ramaiah (**OP 118**) indicated that his bFGF peptide lotion which is marketed in India can be used in combinatorial protocols.

Discussion:

Indications for surgical treatment: Several panellists warned against such therapies in patients who are not clearly stabilized. Surgery on hands needs immobilization which is not easy to obtain in practice.

Non melanoma and melanoma skin cancer and UV exposure in vitiligo patients. There is no demonstrated increased risk from UV in vitiliginous skin (and the contrary is suggested based on anecdotal reports in tropical countries) and UVBTL01 treatment is considered as a safe first-line therapy in vitiligo. There is no need to avoid natural sunlight, since UVB boosts melanocyte division and migration. UVA may act indirectly on the melanocyte environment, through growth factor production by epidermal and dermal cells, promoting melanocyte survival and pigmentation. A moderate suberythemal exposure (that is, less exposure than leads to sunburn) is advised to enhance repigmentation, followed by sun protection if further exposure cannot be avoided (summary of various panellists' answers). It is often assumed that skin is protected against sunburn predominantly by melanin. However, Dr Westerhof mentioned a difference in burning capacity of white patches between vitiligo individuals with different skin types. With UVB 311 nm lamps, he irradiated both lesional and non-lesional skin with increasing doses in 33 patients with vitiligo, divided into 5 groups according to skin type (II-VI). Twenty-four hours later he assessed the minimal erythemal dose and found a correlation between skin type and UV sensitivity in both lesional skin and normal skin. He suggests that there must be a protection mechanism, other than that offered by melanin pigmentation. Antioxidant status may play a role in this phenomenon[10].

UV treatments in children: The benefit/risk ratio is frequently evaluated with a strong negative bias in children because the potential side effects of treatments are overemphasized. However, the benefit of an early stabilizing treatment is currently considered more important than the risk of UV irradiation. The limiting factor is the practical management of UVBTL01 therapy in a child, which is generally possible only around the age of 6-7 or older.[11]

[8] Chen YF, Yang PY, Hu DN, Kuo FS, Hung CS, Hung CM. Treatment of vitiligo by transplantation of cultured pure melanocyte suspension: analysis of 120 cases. J Am Acad Dermatol. 2004;51:68-74.

[9] Yu HS, Wu CS, Yu CL, Kao YH, Chiou MH. Helium-neon laser irradiation stimulates migration and proliferation in melanocytes and induces repigmentation in segmental-type vitiligo.J Invest Dermatol. 2003;120:56-64.

[10] Caron-Schreinemachers AL, Kingswijk MM, Bos JD, Westerhof W. UVB 311 nm tolerance of vitiligo skin increases with skin phototype. Acta Derm Venereol. 2005;85:24-6.

[11] Atherton DJ, Cohen BL, Knobler E, Garzon M, Morelli JG, Tay YK, Weston WL, Taieb A, Morison WL, Rasmussen JE. Phototherapy for children. Pediatr Dermatol. 1996;13:415-26.

Session III: New directions for research (Interface between clinical and basic research)
Chairs: C Goding (Oxted, UK), A Taïeb (Bordeaux, France)
Panelists: L Larue (Orsay, France), D Bennett (London, UK), P Das (Amsterdam, NL), R Spritz (Denver, USA)

Non immune and immune pathomechanisms were respectively introduced by Mauro Picardo (Rome, Italy) and Caroline LePoole (Chicago, USA).

Dr Picardo reviewed primary cellular defects and alterations of the melanocyte microenvironment that can lead to the disappearance of functional melanocytes, and considered auto-immune phenomena as secondary. He made the point that vitiligo is probably not a single disease and that it may correspond to multiple causes. He examined neural, metabolic, genetic, redox and adhesion dependent (melanocytorrhagic[12]) mechanisms. Melanogenic and extra-melanogenic metabolism, including for the latter catecholamines, calcium, antioxidant, and pterins, have all been shown to be altered to some extent in vivo or in vitro. A clear genetic basis for these alterations is not yet at hand. He made the hypothesis that altered gene expression could affect the amount or the correct folding of proteins involved in the synthesis of melanin or in the detoxifying process, with subsequent increased melanocyte vulnerability. He emphasized the current evidence for a compromised intracellular redox status due to both impaired antioxidant defence and increased free radical production. The detachment of melanocytes could be the net effect of convergent pathways altering melanocyte survival and give rise to secondary autoimmune responses.

Dr LePoole stated that in vitiligo depigmentation is accompanied by T cell influx to the skin in the vast majority of patients, in an entity she designated as “auto-immune vitiligo”. A minority of such infiltrating T cells are type 1 proinflammatory cytokine-secreting cells reactive with melanocyte-specific antigen. Melanoma research has shown that differentiation antigens, also expressed by normal melanocytes, can be immunogenic when expressed in the melanosomal compartment of the cell. Similar reactivity to melanosomal antigens is apparent for T cells infiltrating vitiligo skin. Stress may be a precipitating factor of the immune response inadequately modulated by regulatory T cells. T cells are recruited to the skin as a function of dendritic cell activation and dendritic cells are likely activated at sites of epidermal trauma as a consequence of stress proteins such as HSP that spill over into the microenvironment. Stress proteins chaperoning antigens representative of the cells from which they were derived are then processed by dendritic cells and contribute to their activation. Activated dendritic cells not only migrate to draining lymph nodes to recruit T cells but may execute cytotoxic effector functions as well. The contribution of the effector functions to actual depigmentation of the skin remains to be investigated.

A first debate was launched based on these two reviews and on the list of questions from the UK Vitiligo Society summarized by Maxine Whitton.

Colin Goding speculated on a common stress signalling pathway hypothesis which may reconcile immune and non-immune pathomechanisms, acting on both keratinocytes which provide survival and growth promoting factors for melanocytes, and of course melanocytes, as well as on various dermal cells which can influence melanocyte behaviour. Another hypothesis concerns alterations in stem cells that could also be influenced by stressor factors. He also underlined the role of the transcription factor MITF in the loss of melanocytes and in depigmentation. The relevance of this mechanism is supported by *in vivo* (mouse with *vit* gene deletion) and *in vitro* studies. The mitogen-activated kinase (MAPK) p38 has been shown to transduce a variety of stress stimuli including UV, mechanical and hormonal stress into cellular responses by phospho-relay cascades, which are possible research targets. One possibility raised is that melanocytes from vitiligo patients are intrinsically more sensitive to stress signalling via the p38 pathway.

[12] Gauthier Y, Cario Andre M, Taieb A. A critical appraisal of vitiligo etiologic theories. Is melanocyte loss a melanocytorrhagy? Pigment Cell Res. 2003;16:322-32.

In conclusion of this debate, Colin Goding summarized his views as follows: vitiligo appears to be a complex disease in which melanocytes are intrinsically stress sensitive, leading most likely to melanocyte death in response to various kinds of stress - mechanical, viral, even emotional. This would then lead to vitiligo only in those patients who also have genetic predisposition to an auto-immune reaction against melanocyte antigens.

Further questions from **Maxine Whitton** were addressed:

Is there an agreement on the nature of pigment loss? Are all the melanocytes in vitiliginous skin dead, or do some survive in the white patches, which can be stimulated to divide and multiply?
This question refers to the staging of the disease and has important therapeutic consequences. However, the panellists present were not enthusiastic to answer it, because there are more opinions than facts on this matter. Repigmentation can occur from hair follicles and sometimes focally on glabrous skin such as lips – so surely most would agree that sometimes melanocytes or their precursors are still present within the patches in this case. We just don't know if they always are. Further research is obviously needed.

Why does vitiligo appears on particular parts of the body? What is different in those parts of the skin or underlying tissues that predisposes one part to manifest depigmentation and not another?
The panellists agreed upon the role of environmental/triggering factors especially trauma causing Koebner's phenomenon, but there are probably other unknown factors. Another point was made by Dr Lionel Larue (Orsay, France) who speculated about the role of melanocyte migration, which makes it take longer for the precursor cells to reach the extremities, so that fewer cells arrive in these areas. This may affect the susceptibility of melanocytes in acral locations. Prof Bennett commented that there was also speculation on a role for neurotransmitters, since some of the susceptible areas (e.g. around the eyes and mouth and the fingers) have a rich nerve supply.

Some people report itching in their vitiligo patches, often it is the precursor to a new white spot. For others the white patches are more sensitive to products such as soap and shampoo. If itching is an inflammatory response then all people with vitiligo should experience it (cf eczema, acne). Has any research been done on itching in vitiligo?
Dr Taieb replied that so-called "inflammatory vitiligo" is a known but rare event and that pruritus is rarely mentioned spontaneously by patients visiting our clinics. This point was agreed upon by other clinical experts. Pruritus would be indeed a good argument for the autoimmune or auto-inflammatory theory of vitiligo. However, it appears important to obtain this information more systematically when taking the patient's history and even to use a pruritus scale in this disease. The VETF should include this item in the updated version of its vitiligo evaluation form.

Five short presentations were discussed in this session.
Dr Thomas Tüting (Bonn, Germany) presented a mouse model using C57BL/6 mice which indicates that CD4 T cell help and local inflammation are required to circumvent peripheral CD8 tolerance against melanocytic antigens. Using two different genetic methods for the induction of cellular immunity in vivo, gene gun bombardment of the skin and injection of recombinant adenovirus, his group has shown that peripheral tolerance of CD8+ T cells recognizing a single TRP2-derived H2-Kb-binding peptide is regulated in two steps. In the induction phase, stimulation and expansion of TRP2-specific CD8+ T cells in vivo depend on CD4+ T cell help. In the effector phase, autoimmune destruction of melanocytes in the skin depends on local inflammation. He suggest that accidental stimulation of CD8+ CTL recognizing major histocompatibility complex class I-binding peptides

derived from melanocytic proteins in the context of an inflammatory skin disease may play an important role in the pathophysiology of vitiligo[13].
This paper raises an issue in line with one of the above questions, and would suggest a more thorough look at inflammatory premises of vitiligo which are so far not clear in most patients.

Dr Silvia Moretti (Florence, Italy), pursuing her previous work on done using immunohistochemistry of cytokine expression[14] described modifications in cytokine transcripts for ET1, SCF , GM-CSF, bFGF and TNF in 12 patients with active NS vitiligo. ET1 and SCF were more expressed in perilesional than lesional skin, whereas GM-CSF and bFGF were more present in lesional than perilesional skin. TNF , which has an inhibiting effect on melanocyte growth and differentiation, was highly expressed in both perilesional and lesional skin, but not detectable in normal control skin. Similar data for TNF transcripts have been reported by Dr Grimes and colleagues[15]. During the discussion Dr Taïeb mentioned that if this finding is relevant, it was surprising that in the large number of patients treated with anti-TNF agents, no cure of vitiligo has been so far mentioned. One case of infliximab related vitiligo has even been published[16]. Dr Grimes mentioned that a study of anti-TNF in vitiligo is under consideration at her institution.

Dr Paola Grammatico (Rome, Italy) took the candidate gene approach for NS vitiligo. She looked at CDKN2C, a tumor suppressor gene located in the AIS1 region recognized as a vitiligo susceptibility locus, at the microphthlalmia (MITF) gene which encodes a transcription factor important for melanocyte survival, and at the angiotensin converting gene (ACE) I/D polymorphisms recently reported in the Korean population in vitiligo patients. The results were negative when using Italian control samples. Dr Richard Spritz commented that candidate gene approaches should not be performed in multigenic disease given their very low yield of positive results. He has developed linkage studies that have been more fruitful.
However, Dr Taïeb pointed out that other complex diseases benefited from the candidate gene approach, when a monogenic disorder is highly associated with the considered phenotype, quoting the example of Netherton syndrome and atopic dermatitis[17].
Dr Muriel Cario-André (Bordeaux, France) summarized the prize winning IPCC poster (**PP 050**) which demonstrates a strong dermal influence on human epidermal pigmentation. Using epidermal reconstructs seeded on various types of dermis, in vitro and in vivo with xenografts on tolerant Swiss nu/nu mice, she showed that dermal fibroblast density/activity influences melanocyte migration and proliferation and possibly melanin distribution and degradation. How this applies to vitiligo and other skin disorders remains elusive but the findings suggest consideration of the dermal influence on the epidermal melanin unit as more important than previously envisaged.

Session IV: Burden of disease/Interaction with patients' support groups
Chair: Alida de Pase (ASNPV, Italy)
Alida de Pase has reported on this Satellite in a separate paper.
The interaction of patients' support groups with the medical and scientific community has already been fruitful as communicated by Richard Spritz at the IPCC, since major predisposing genes have been detected in the families contacted in the UK and North America by patient support groups. It was also

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[13] Steitz J, Bruck J, Lenz J, Buchs S, Tuting T. Peripheral CD8+ T cell tolerance against melanocytic self-antigens in the skin is regulated in two steps by CD4+ T cells and local inflammation: implications for the pathophysiology of vitiligo. J Invest Dermatol. 2005;124:144-50.
[14] Moretti S, Spallanzani A, Amato L, Hautmann G, Gallerani I, Fabiani M, Fabbri P. New insights into the pathogenesis of vitiligo: imbalance of epidermal cytokines at sites of lesions. Pigment Cell Res. 2002;15:87-92.
[15] Grimes PE, Morris R, Avaniss-Aghajani E, Soriano T, Meraz M, Metzger A. Topical tacrolimus therapy for vitiligo: therapeutic responses and skin messenger RNA expression of proinflammatory cytokines. J Am Acad Dermatol. 2004;51:52-61.
[16] Ramirez-Hernandez M, Marras C, Martinez-Escribano JA.Infliximab-induced vitiligo. Dermatology. 2005;210:79-80.
[17] Walley AJ, Chavanas S, Moffatt MF, Esnouf RM, Ubhi B, Lawrence R, Wong K, Abecasis GR, Jones EY, Harper JI, Hovnanian A, Cookson WO. Gene polymorphism in Netherton and common atopic disease. Nat Genet. 2001;29:175-8.

important to have the personal account of the patients and they deputized brilliant speakers at the satellite meeting. Randy Salter from Vitiligo Support International reported on his personal experience, and how difficult it was in general to find doctors with an interest in vitiligo patients. A dePase also made a practical point during the meeting concerning paediatric patients, who should be seen in separate clinics because of losing all hope when mixed up with affected adults in the same waiting room. The AVRF (American Vitiligo Research Foundation) deputized Roxanne Knight and Marilyn Giordano who presented slides of severely affected children, bringing home to the audience the message of how urgent it was for the medical/scientific community to address the problem of vitiligo.

Conclusions

Mauro Picardo and Alain Taieb expressed their thanks to all the speakers and participants and delineated some future steps. The Special Interest Group on Vitiligo of the IFPCS should have a mailing list to communicate more easily on the internet, and receive messages concerning future initiatives. A specific website would be helpful. A vitiligo meeting will be held at the next ESPCR meeting in Barcelona organized by Luis Montoliu (24-27 Sept 2006) and at the next IPCC in Sapporo, Japan, May 7-12, 2008. Exchanges should be improved to promote an international agreement on such basic issues as assessment tools and outcome measures in clinical trials. Fostering exchanges between the clinical and scientific communities around vitiligo will be a priority and research projects could be set up based on some ideas debated at this Symposium. Fund raising for research is another important issue and the help of patient support groups is expected.

Appendix I: List of IPCC papers and posters related to vitiligo research

OP 2, 25, 35, 38, 74-77, 116, 117, 119-124
KL 5
PL 11-12
PP 33B, 33C, 36, 46, 47, 49-54, 56, 60A, 60C, 65

Appendix II: VETF assessment form

CURRENT LITERATURE

1. Chemistry of Melanins and other Pigments

(Dr. A. Napolitano)

Several studies have appeared reporting the properties of natural and synthetic melanins. The binding to melanins of drugs with different structural characteristics were studied (Bridelli et al, *Biophys. Chem*) and the parameters allowing to define drug surface interaction were obtained. A quantitative analysis of the fluorescence spectra of synthetic eumelanins was reported together with three dimensional maps of the specific quantum yields (Nighswander-Rempel et al, *J. Chem Phys*).

Further studies by Meredith's group in Brisbane on eumelanins focus on the oxidation of 5,6-dihydroxyindole-2-carboxylic acid performed in alkaline conditions. The absorbance properties of the final oxidation mixture is interpreted in terms of the chemical disorders model according to which the broadband monotonic absorption characteristic of all melanins is a consequence of the superposition of a large number of nonhomogeneously broadened Gaussian transitions associated with each of the components of a melanin ensemble. Density function theory (DFT) calculations were applied to a number of oligomers of 5,6-dihydroxyindole-2-carboxylic acid most of which, however, have hypothetic structures with two positions of each indole unit involved in the linking; the results showed HOMO-LUMO gaps in the oligomers red-shifted with respect to the monomers. (Tran et al., *Biophys. J*)

Of interest is also the study of the spectral properties of trichochromes, the the $\Delta^{2,2'}$-bi(2*H*-1,4-benzothiazine) associated to pheomelanin pigments (Simon et al *Photochem. Photobiol.*). The theroretical absorption spectra obtained substantially confirmed previous data on the relative stability of the cis and trans isomer at the central double bond, and the equilibrium of the keto-enol forms. The photoreactivity of trichochromes and their ability to act as quenchers of singlet oxygen were also investigated.

A variety of melanogenesis inhibitors were reported which range from synthetic products such as kojic acid derivatives (Lee et al *Arch Pharm*), amides obtained from coupling of *p*-cinnamic acid derivatives with phenylalkylamines (Okombi et al, *Bioorg. Med. Chem. Lett.*), and selenium-containing carbohydrates (Ahn et al *Chem. Pharm. Bull*) to natural products such as cycloartane type triterpenoids derived from Amberboa ramose Jafri (Khan et al *Bioorg. Med. Chem*), ellagic acid in pomegranate extracts (Yoshimura et al *Biosci. Biotechnol. Biochem*) and plant derived phenylpropoanoids (Tanimoto et al *Yakugaku Zasshi*). The mechanism of the inhibitory action of 3,4-dihydroxyacetophenone (Kim et al. *Biosci. Biotechnol. Biochem.*), 4,4'-dihydroxybiphenyl (No et al. *Biol. Pharm. Bull*) and 4-*n*-butylresorcinol (Kim et al. *Biol. Pharm. Bull*) was also investigated.

Determination of eumelanin in skin biopsies by identification and quantitation of PTCA based on LC/MS/MS methodology was reported by a group at Pfizer Global (Szekely-Kleper et al *J. Chromatogr.*). A study by Ito's group developed a method to measure levels of eumelanin in urine samples and evaluate its clinical significance in comparison with the melanin-related metabolites 6-hydroxy-5-methoxyindole-2-carboxylic acid and 5-S-cysteinyldopa, and with pheomelanin, measured after degradation as 4-amino-3-hydroxyphenylalanine (Wakamatsu et al *Pigm. Cell Res*.)

REACTIVITY AND PROPERTIES

- Albuquerque JE, Giacomantonio C, White AG, Meredith P.
  Study of optical properties of electropolymerized melanin films by photopyroelectric spectroscopy. Eur. Biophys. J35(3), 190-195, 2006.

- Bridelli MG, Ciati A, Crippa PR.
  Binding of chemicals to melanins re-examined: Adsorption of some drugs to the surface of melanin particles. Biophysical Chemistry 119(2), 137-145, 2006.

- Haywood RM, Lee M, Linge C.
  Synthetic melanin is a model for soluble natural eumelanin in UVA-photosensitised superoxide production. J. Photochem. Photobiol, B. 82(3), 224-235, 2006.

- Nighswander-Rempel SP, Riesz J, Gilmore J, Meredith P.
  A quantum yield map for synthetic eumelanin. J Chem Phys. 123(19), 19*4901, 2005.*

- Rizzi A, Comai S, Bertazzo A, Costa CV, Allegri G, Traldi P.
  An investigation on the possible role of melatonin in melanogenesis. J Mass Spectrom. 2006, Feb 23.

- Sarangarajan R, Apte SP.
The polymerization of melanin: a poorly understood phenomenon with egregious biological implications. Melanoma Research 16(1), 3-10, 2006.

- Simon J D, Goldsmith MR, Hong L, Kempf VR, McGuckin LEL, Ye T, Zuber G.
Spectroscopy and photoreactivity of trichochromes: molecular components of pheomelanins. Photochem. Photobiol. 82, 318-323, 2006.

- Tran ML, Powell BJ, Meredith P.
Chemical and structural disorder in eumelanins: a possible explanation for broadband absorbance. Biophys. J 90(3), 743-752, 2006.

- Wang Z, Dillon J, Gaillard ER.
Antioxidant Properties of Melanin in RPE cells. Photochem Photobiol. 2005 Oct 1.

BIOSYNTHESIS / CONTROL

- Ahn SJ, Koketsu M, Ishihara H, Lee SM, Ha SK, Lee KH, Khang TH, Kima SY.
Regulation of melanin synthesis by selenium-containing carbohydrates. Chem. Pharm. Bull. (Tokio), 54(3), 281-286, 2006.

- Khan MTH, Khan SB, Ather A.
Tyrosinase inhibitory cycloartane type triterpenoids from the methanol extract of the whole plant of Amberboa ramosa Jafri and their structure-activity relationship. Bioorg. Med. Chem. 14(4), 938-943, 2006.

- Kim DS, Kim SY, Park SH, Choi YG, Kwon SB, Kim MK, Na JI, Youn SW, Park KC.
Inhibitory effects of 4-n-butylresorcinol on tyrosinase activity and melanin synthesis. Biol Pharm Bull. 28(12), 2216-2219, 2005.

- Kim YJ, No JK, Lee JS, Kim MS, Chung HY.
Antimelanogenic Activity of 3,4-Dihydroxyacetophenone: Inhibition of Tyrosinase and MITF. Biosci Biotechnol Biochem. 70(2), 532-534, 2006.

- Lee YS, Park JH, Kim MH, Seo SH, Kim HJ.
Synthesis of Tyrosinase Inhibitory Kojic Acid Derivative. Arch Pharm (Weinheim). 339(3):111-114, 2006.

- Nerya O, Ben-Arie R, Luzzatto T, Musa R, Khativ S, Vaya J.
Prevention of Agaricus bisporus postharvest browning with tyrosinase inhibitors. Postharvest Biol. Technol. 39(3), 272-277, 2006.

- No JK, Kim YJ, Lee JS, Chung HY.
Inhibition of melanogenic activity by 4,4'-dihydroxybiphenyl in melanoma cells. Biol. Pharm. Bull. 29(1), 14-16, 2006.

- Okombi S, Rival D, Bonnet S, Mariotte AM, Perrier E, Boumendjel A**.**
Analogues of N-hydroxycinnamoylphenalkylamides as inhibitors of human melanocyte-tyrosinase. Bioorg Med Chem Lett. 16(8), 2252-2255, 2006.

- Tanimoto S, Tominaga H, Okada Y, Nomura M.
Synthesis and cosmetic whitening effect of glycosides derived from several phenylpropanoids. Yakugaku Zasshi,. 126(3), 173-177, 2006.

- Yoshimura M, Watanabe Y, Kasai K, Yamakoshi J, Koga T.
Inhibitory effect of an ellagic acid-rich pomegranate extract on tyrosinase activity and ultraviolet-induced pigmentation. Biosci Biotechnol Biochem. 69(12), 2368-2373, 2005.

MELANIN ANALYSIS

- Szekely-Klepser G, Wade K, Woolson D, Brown R, Fountain S, Kindt E.

A validated LC/MS/MS method for the quantification of pyrrole-2,3,5-tricarboxylic acid (PTCA), a eumelanin specific biomarker, in human skin punch biopsies. J. Chromatography, B. 826(1-2), 31-40, 2005.

- Wakamatsu K, Kavanagh R, Kadekaro AL, Terzieva S, Sturm RA, Leachman S, Abdel-Malek Z, Ito S.
  Diversity of pigmentation in cultured human melanocytes is due to differences in the type as well as quantity of melanin. Pigment Cell Res. 19(2), 154-62, 2006.

- Wakamatsu K, Takasaki A, Kagedal B, Kageshita T, Ito S.
  Determination of eumelanin in human urine. Pigment Cell Res. 19(2), 163-9, 2006.

OTHER PIGMENTS

- Cunha MM, Franzen AJ, Alviano DS, Zanardi E, Alviano CS, De Souza W, Rozental S.
  Inhibition of melanin synthesis pathway by tricyclazole increases susceptibility of Fonsecaea pedrosoi against mouse macrophages. Microsc. Res. Tech. 68(6), 377-84, 2005.

- da Silva MB, Marques AF, Nosanchuk JD, Casadevall A, Travassos LR, Taborda CP.
  Melanin in the dimorphic fungal pathogen Paracoccidioides brasiliensis: effects on phagocytosis, intracellular resistance and drug susceptibility. Microbes Infect. 8(1), 197-205, 2006.

- Frases S, Chaskes S, Dadachova E, Casadevall A.
  Induction by Klebsiella aerogenes of a melanin-like pigment in Cryptococcus neoformans. Appl. Environ. Microbiol. 72(2), 1542-1550, 2006.

- Wang H, Pan Y, Tang X, Huang Z.
  Isolation and characterization of melanin from Osmanthus fragrans' seeds. LWT-Food Sci. Technol. 39(5), 496-502, 2006.

2. Biology of pigment cells and pigmentary disorders

(Dr. M. Picardo)

Several studies have been focused on the regulatory role of Mitf in the melanocytes. Among the pathways melanocytes employ to maintain growth and survival, **McGill et al** evaluated c-Met receptor tyrosine kinase, a multifaceted regulator of growth, motility and invasion in a number of lineages *in vivo*. Considering that c-Met and its ligand Scatter factor /Hepatocyte Growth (HGF) have been suggested to play a role of in the development regulation of melanocytes and that a number of key melanocyte pathways, such as c-Kit, α-MSH, Wnt, and endothelin appear to converge on the master lineage regulator Mitf, the authors investigated whether HGF/c-Met may transduce some of its downstream signalling through Mitf. Their results show that :1) stimulation of primary human melanocytes and human melanoma cells with HGF was able to induce phosphor-Met, followed by MAPK and Mitf phosphorylation and degradation, and finally increased c-Met message and protein; 2) *c-met* is a direct transcriptional target of mitf in human melanocytes; 3) the molecular events and kinetics of signalling downstream of HGF is indistinguishable from those induced by SCF/c-kit signalling, indicating that Mitf phosphorylation results in a coupled activation/degradation mechanism; 4) inhibition of Mitf blocked HGF-mediated invasion, suggesting that Mitf regulates key intermediates required for matrix invasion. **Hoogdujin and co-workers** examined the possible regulatory role of glutamate signalling system in normal human melanocytes. They found that : 1) ionotropic glutamate receptors, such as GluR2, GluR4, NMDARA, and NMDARC, are expressed in melanocytes, suggesting an active transport of glutamate in these cells; 2) the glutamate receptors on melanocyte respond via an intracellular calcium increase in response to AMPA and to a lesser extent NMDA; 3) in contrast with its reported effects in neurones, L-glutamate do not show any evidence of toxicity in melanocytes; 4) melanocytes appear neither to produce nor to release L-glutamate; 5) L-glutamate treatment do not induce melanin production in melanocytes; 6) blockage of AMPA receptors caused a rapid and reversible change in melanocyte morphology, together with a reduced expression of Mitf, indicating that glutamate signalling is involved in Mitf gene expression. These data suggest that alteration of glutamate signalling can lead to opposite melanocyte disorders. The morphology changes of melanocytes, accompanied by reduction of their differentiation and their capacity to produce melanin can reproduce the de-pigmentation disorder vitiligo, whereas reduction of MiTF can lead to melanoblast hyperproliferation, suggesting that disturbed glutamate signalling can be involved in the development of melanoma. **Park et al** investigated whether cAMP regulates the expression of PKC-β, the kinase responsible for activating tyrosinase. Due to the fact that the promoter region of PKC-β contains at least two E-boxes, the authors explored also the possible role of MITF-M as a transcription factor for PKC-β. Their data demonstrate that the expression of PKC-β is up-regulated by cAMP via the key transcription factor MITFM, indicating the existence of a coordinate regulation of multiple melanogenic proteins in melanocytes and suggesting that the PKC and cAMP pathways interact in the regulation of mammalian pigmentation. To investigate the expression of PKC isoforms in human epidermal melanocytes in situ, **Oka and co-workers** performed immunofluorescent analysis of human neonatal foreskin. They find that almost all cells stained with Mel-5, a monoclonal antibody reacting with tyrosinase-related protein-1, reacted with monoclonal antibodies to PKCα, PKCβ and PKCδ indicating that these isoforms are expressed in human epidermal melanocytes in situ. **Schallreuter et al** presented evidence that human epidermal melanocytes transcribe and translate both methionine sulfoxide reductases (methionine sulfoxide reductase A, which reduces methionine S-sulfoxide, and methionine sulfoxide reductase B, which reduces the R-diastereomer) as well as thioredoxin reductase in their cytosol and in the nucleus. These data can indicate that melanocytes are able to protect their methionine proteins against ROS induced stress, through the activation of the thioredoxin reductase/methionine S-R sulfoxide reductases cascades as repair mechanisms for methionine sulfoxide formation. **Bowan and co-workers** reported that keratin 16 is constitutively expressed by all melanocytes. These data are in contrast with prevailing dogma stating that keratin filaments are the hallmark of keratinocytes and other epithelial cells, indicating that these cytoskeleton components may play important roles in tissues other than epithelia. To identify the hitherto unknown melanocyte-specific genes, **Takeda et al,** using a cDNA arrays, compared mRNA expression profiles between wild-type and $Mitf^{mi\text{-}bw}$ homozygous mouse skin. They provide several lines of evidence that lipocain-type prostaglandin D synthase (L-PGDS), which catalyzes the isomeration of prostaglandin (PG) H_2 to produce PGD_2, represents a newly identified melanocyte marker regulated by MITF. **Mischiati and co-workers** compared cDNA, profiled by macroarray and RT-PCR, in paired sets of melanoma and melanocytes from metastatic lesions and the uninvolved skin of three patients. They showed several gene products up-regulated of down-regulated in at least 1 pair, but only 3 (STAT2, collagen type VI, and CD9) were concordantly down-modulated in all 3 pairs. Considering that the three gene products were down-regulated at different stages of melanoma progression, the authors suggest that cDNA profiling of paired melanocyte/melanoma cultures can reveal novel, early signatures of melanocyte transformation. Some studies provided new evidence on possible mechanisms and stimuli capable of inducing apoptosis in melanocytes. The correlation between activation of peroxisome proliferator-activated receptors (PPARs), which are members of nuclear hormone receptor superfamily, expressed in melanocytes and involved in lipid metabolism, melanocyte growth and apoptosis have been investigated by **Kang et al.** In their study the treatment with ciglitazone, a PPAR-gamma activator, inhibits growth of human melanocytes by inducing apoptosis, with a mechanism involving a time-dependent Bcl-2 protein reduction and caspase-3 protein increase. **Bivik and coworkers** demonstrated UVA/B to induce apoptosis in human melanocytes through the mitochondrial pathway, displaying cytochrome c release, caspase-3 activation, and fragmentation of nuclei. Their evidence indicate that Bcl-2 family play a central role in the outcome of the death signal induced by UV in melanocytes, and the lysosomal proteases, cathepsin B and D, which may act as proapoptotic

mediators, are involved in translocation of Bax from the cytosol to punctate mitochondrial-like structures. **Kulesz-Martin et al** analysed melanocytes and keratinocytes for the potential role of p53, p73, and p63 tumor suppressor family proteins and of malignancy-specific gene expression changes in the etiology of multi-step cancer. The influence of epidermis microenvironment in altering melanocyte homeostasis and potentially leading to their transformation in tumoral cells, have been extensively reviewed by **Haass and Herlyn**. The authors described downregulation of receptors involved in communication with keratinocytes, such as E-cadherin, P-cadherin, and desmoglein, up-regulation of receptors and signalling molecules mediating melanoma-melanoma cell and melanoma-fibroblast cell interaction, such as N-cadherin and Mel-CAM, deregulation of morphogens and their ligands, loss of anchorage of to the basement membrane, and increased expression of metalloproteinases, as the five major mechanisms by which melanoma cells escape form control by keratinocytes, hyperproliferating and invading surrounding tissues. **Seftor and co-workers** developed a three dimensional in vitro model for investigating the molecular changes that occur during melanocytic progression to a melanoma phenotype. They investigated the behaviour of normal melanocytes interacting with a metastatic melanoma matrix to determine whether tumor cell environment is able to induce the epigenetic trans-differentiation of the normal melanocyte phenotype to that of an aggressive melanoma-like cell. **Passeron and Ortonne** reviewed the main hypothesis for physiopathology of vitiligo, in particular focusing on the autoimmune pathogenesis of vitiligo and candidate genes for vitiligo susceptibility. Due to the fact that neural vitiligo hypothesis involves increased release of norepinefrine, a melanocytotoxin, from the autonomic nerve and subsequent injure of melanocytes, **Namazi** proposed phenytoin, a well-known anticonvulsant, in the treatment of vitiligo. Phenytoin is suggested to inhibits the release of norepinefrine and its induction of the catecholamine degrading enzyme monoamine oxidase (MAO). Moreover the drug seemed to interact with membrane lipids, thus promoting stabilization of membranes and preventing the diffusion of toxic melanin precursor into the cytoplasm. These evidence can support the hypothesis that phenytoin could represent a therapeutically treatment effective against vitiligo.

- Bivik CA. Larsson PK, Kagedal KM, Rosdahl IK, Ollinger KM.
  UVA/B-induced apoptosis in human melanocytes involves translocation of cathepsins and bcl-2 family members. Mar, [Epub ahead of print], 2006.

- Bhawan J, Whren K, Panova I, Yaar, M.
  Keratin 16 expression in epidermal melanocytes of normal human skin. Am J Dermatopathol, 27: 476-481, 2006.

- Haas NK, Herlyn M.
  Normal human melanocyte homeostasis as a paradigm for understanding melanoma. J Invest Dermatol Symp Proc, 10: 153-163, 2005.

- Hoogdujin MJ, Hitchcock IS, Smit NP, Gillbro JM, Schallreuter KU, Genever PG.
  Glutamate receptors on human melanocytes regulate the expression of MiTF. Pigment Cell Res, 19: 58-67, 2006.

- Kang HY, Lee JY, Lee JS, Choi YM.
  Peroxisome proliferator-activated receptors-gamma activator, ciglitazone, inhibits human melanocyte-growth trough induction of apoptosis. Arch Dermatol Res, Feb 11 [Epub ahead of print], 2006.

- Kulesz-Martin M, Lagowski J, Fei S, Pelz C, Sears R, Powell MB, Halaban R, Johnson J.
  Melanocyte and keratinocyte carcinogenesis: p53 family protein activities and intersecting mRNA expression profiles. J Invest Dermatol Symp Proc, 10: 142-152, 2005.

- Johnson J, Lagowski J, Sunderberg A, Kulesz-Martin M.
  P53 family activities in development and cancer: relationship to melanocyte and keratinocyte carcinogenesis. J Invest Dermatol, 125: 857-864, 2005.

- McGill GG, Haq R, Nishimura EK, Fisher DE.
  c-met expression is regulated by mitf in the melanocyte lineage. J Biol Chem, Feb 2 [Epub ahead of print], 2006.

- Mischiati C, Natali PG, Sereni A, Sibilio L, giorda E, Cappellacci S, Nicotra MR, Mariani G, Di Filippo F, Catricalà C, Gambari R, Grammatico P, Giacobini P.
  cDNA-array profiling of melanomas and paired melanocyte cultures. J Cell Physiol, Mar 7 [Epub ahead of print], 2006.

- Namazi MR.
  Phenytoin as a novel anti-vitiligo weapon. J Autoimmune Dis, 22: 2-11, 2005.

- Oka m, Nishigori C, Kageshita T, Hsu MY, Penmatcha S, Herlyn M.
  Expression of PKC isoforms in human melanocytic cells in situ. J Dermatol Sci, 41: 157-161, 2006.

- Park HY, Wu C, Yonemoto L, Murphy-Smit M, Wu H, Stachur CM, Gilchrest BA.
MITF mediates c-AMP-induced PKC-beta expression in human melanocytes. Biochem J, Jan 17 [Epub ahead of print], 2006.

- Passeron T, Ortonne JP.
Physiopathology and genetics of vitiligo. J Autoimmun, 25 Suppl: 63-68, 2005.

- Schallreuter KU, Rubsam K, Chavan B, Zothner C, Gillbro JM, Spencer JD, Wood JM.
Funtioning methionine sulfoxide reductase A and B are present in human epidermal melanocytes in the cytosol and in the nucleus. Biochem Biophys res Commun, 342: 145-152, 2006.

- Seftor EA, Brown KM, Chin L, Kirschmann DA, Wheaton WW, Protopopov A, Feng B, Balagurunathan Y, Trent JM, Nickoloff BJ, seftor RE, Hendrix MJ.
Epigenetic transdiffentiation of normal melanocytes by a metastatic melanoma microenvironment. Cancer Res, 65: 10164-10169, 2005.

- Takeda K, Yokowama S, Aburatani H, Masuda T, Han F, Yoshizawa M, Yamaki N, Yamamoto H, Eguchi N, Urade Y, Shibahara S.
Lipocalin-type prostaglandin D synthase as a melanocyte marker regulated by MITF. Biochem Biophys Res Commun, 339: 1098-1106, 2006.

3. MSH, MCH, other hormones, differentiation

(Dr. R. Morandini)

Garcia-Borron (Pigment Cell Res. 2005) has written an interesting review about Melanocortin-1 receptor structure and functional regulation. In a few words, it has been established that MC1R cDNA is a major determinant of skin and hair pigmentation, sun sensitivity and susceptibility to skin cancer. The functional behavior of the MC1R agrees with emerging concepts in G-protein-coupled receptors signaling including dimerization, coupling to more than one signaling pathway and a high agonist-independent constitutive activity accounting for inverse agonism phenomena. In addition, MC1R displays unique properties such as an unusually high number of natural variants often associated with clearly visible phenotypes and the occurrence of endogenous peptide antagonists. Furthermore, the authors review current knowledge of MC1R structure and function, with emphasis on information gathered from the analysis of natural variants.
Lee (J Gene Med. 2006) and Wang (Gene Ther. 2006) have demonstrated that alpha-melanocyte-stimulating hormone (alpha-MSH) gene therapy protects against thioacetamide-induced acute liver failure and possesses anti-hepatic fibrogenic effect in mice. Alpha-MSH gene therapy hormone expression plasmid was delivered via electroporation. Alpha-MSH gene therapy may be an effective therapeutic modality.
Alpha-MSH can prevent NF-kappaB activation. This leads to a reduction of pro-inflammatory mediators and the inhibition of adhesion molecule expression, with subsequent reduction in leukocyte migration. The development of selective ligands with an appropriate pharmacokinetic profile will enable a pharmacological evaluation of the potential beneficial effects of the melanocortins (Getting SJ. Pharmacol Ther. 2006).
Melanogenesis and melanosome transfer from the melanocytes to the neighboring keratinocytes are induced by ultraviolet radiation and modulated by autocrine and paracrine factors. Using immunofluorescence analysis with an anti-human cytokeratin antibodies and phagocytic assays using fluorescent latex beads Cardinali G at al (J Invest Dermatol. 2005) have investigated the effect of Keratinocyte growth factor on melanosome transfer to keratinocytes. Furthermore an increased expression of the KGF receptor (KGFR) on the keratinocytes by transfection led to increased phagocytosis of latex beads following KGF treatment, suggesting that the KGF effect is directly mediated by KGFR expression and activation. In conclusion Keratinocyte growth factor can promote melanosome transfer to keratinocytes.

Regulation and signal transduction

- Eves PC, MacNeil S, Haycock JW.
  alpha-Melanocyte stimulating hormone, inflammation and human melanoma. Peptides. 27(2):444-52, 2006.

- Fisher A, Mann A, Verma V, Thomas N, Mishra RK, Johnson RL.
  Design and synthesis of photoaffinity-labeling ligands of the L-prolyl-L-leucylglycinamide binding site involved in the allosteric modulation of the dopamine receptor. J Med Chem. 49(1):307-17, 2006.

- Fry D, Dayton B, Brodjian S, Ogiela C, Sidorowicz H, Frost LJ, McNally T, Reilly RM, Collins CA.
  Characterization of a neuronal cell line expressing native human melanin-concentrating hormone receptor 1 (MCHR1). Int J Biochem Cell Biol. 2006 Feb 17.

- Garcia-Borron JC, Sanchez-Laorden BL, Jimenez-Cervantes C.
  Melanocortin-1 receptor structure and functional regulation. Pigment Cell Res. 18(6):393-410, 2005.

- Getting SJ.
  Targeting melanocortin receptors as potential novel therapeutics. Pharmacol Ther. 2006 Feb 16.

- Hill RP, MacNeil S, Haycock JW.
  Melanocyte stimulating hormone peptides inhibit TNF-alpha signaling in human dermal fibroblast cells. Peptides. 27(2):421-30, 2006.

- Hogan K, Peluso S, Gould S, Parsons I, Ryan D, Wu L, Visiers I.
  Mapping the binding site of melanocortin 4 receptor agonists: a hydrophobic pocket formed by I3.28(125), I3.32(129), and I7.42(291) is critical for receptor activation. J Med Chem. 49(3):911-22, 2006.

- Kelly JM, Moir AJ, Carlson K, Yang Y, MacNeil S, Haycock JW.
  Immobilized alpha-melanocyte stimulating hormone 10-13 (GKPV) inhibits tumor necrosis factor-alpha stimulated NF-kappaB activity. Peptides. 27(2):431-7, 2006.

- Kim KS, Kim JA, Eom SY, Lee SH, Min KR, Kim Y.
  Inhibitory effect of piperlonguminine on melanin production in melanoma B16 cell line by downregulation of tyrosinase expression. Pigment Cell Res. 19(1):90-8, 2006.

- Lechan RM, Fekete C.

Role of melanocortin signaling in the regulation of the hypothalamic-pituitary-thyroid (HPT) axis. Peptides. 27(2):310-25, 2006.

- Leder EH, Silverstein JT.
The pro-opiomelanocortin genes in rainbow trout (Oncorhynchus mykiss): duplications, splice variants, and differential expression. J Endocrinol188(2):355-63, 2006.

- Manna SK, Sarkar A, Sreenivasan Y.
Alpha-melanocyte-stimulating hormone down-regulates CXC receptors through activation of neutrophil elastase. Eur J Immunol. 36(3):754-69, 2006.

- Muchmore SW, Souers AJ, Akritopoulou-Zanze I.
The use of three-dimensional shape and electrostatic similarity searching in the identification of a melanin-concentrating hormone receptor 1 antagonist. Chem Biol Drug Des. 67(2):174-6, 2006.

- Murray JF, Hahn JD, Kennedy AR, Small CJ, Bloom SR, Haskell-Luevano C, Coen CW, Wilson CA.
Evidence for a stimulatory action of melanin-concentrating hormone on luteinising hormone release involving MCH1 and melanocortin-5 receptors. J Neuroendocrinol. 18(3):157-67, 2006.

- Palani A, Shapiro S, McBriar MD, Clader JW, Greenlee WJ, O'Neill K, Hawes B.
Biaryl diamides as potent melanin concentrating hormone receptor 1 antagonists. Bioorg Med Chem Lett. 15(23):5234-6, 2005.

- Roberts DW, Newton RA, Beaumont KA, Helen Leonard J, Sturm RA.
Quantitative analysis of MC1R gene expression in human skin cell cultures. Pigment Cell Res.19(1):76-89, 2006.

- Ulven T, Little PB, Receveur JM, Frimurer TM, Rist O, Norregaard PK, Hogberg T.
6-Acylamino-2-amino-4-methylquinolines as potent melanin-concentrating hormone 1 receptor antagonists: structure-activity exploration of eastern and western parts. Bioorg Med Chem Lett. 16(4):1070-5, 2006.

- Xu R, Li S, Paruchova J, McBriar MD, Guzik H, Palani A, Clader JW, Cox K, Greenlee WJ, Hawes BE, Kowalski TJ, O'neill K, Spar BD, Weig B, Weston DJ.
Bicyclic[4.1.0]heptanes as phenyl replacements for melanin concentrating hormone receptor antagonists. Bioorg Med Chem. 2006 Jan 25.

Global effect on cell *in vitro*

- Cardinali G, Ceccarelli S, Kovacs D, Aspite N, Lotti LV, Torrisi MR, Picardo M.
Keratinocyte growth factor promotes melanosome transfer to keratinocytes. J Invest Dermatol. 125(6):1190-9, 2005.

- Gatti S, Carlin A, Sordi A, Leonardi P, Colombo G, Fassati LR, Lipton JM, Catania A.
Inhibitory Effects of the Peptide (CKPV)(2) on Endotoxin-Induced Host Reactions. J Surg Res. 131(2):209-14, 2006.

- Kanuma K, Omodera K, Nishiguchi M, Funakoshi T, Chaki S, Nagase Y, Iida I, Yamaguchi JI, Semple G, Tran TA, Sekiguchi Y.
Identification of 4-amino-2-cyclohexylaminoquinazolines as metabolically stable melanin-concentrating hormone receptor 1 antagonists. Bioorg Med Chem. 2006 Jan 21.

- Liu GS, Liu LF, Lin CJ, Tseng JC, Chuang MJ, Lam HC, Lee JK, Yang LC, Chan JH, Howng SL, Tai MH.
Gene transfer of pro-opiomelanocortin prohormone suppressed the growth and metastasis of melanoma: involvement of alpha-melanocyte-stimulating hormone-mediated inhibition of the nuclear factor kappaB/cyclooxygenase-2 pathway. Mol Pharmacol. 69(2):440-51, 2006.

- Muceniece R, Zvejniece L, Liepinsh E, Kirjanova O, Baumane L, Petrovska R, Mutulis F, Mutule I, Kalvinsh I, Wikberg JE, Dambrova M.
The MC(3) receptor binding affinity of melanocortins correlates with the nitric oxide production inhibition in mice brain inflammation model. Peptides. 2006 Jan 13.

- Sabatier N, Leng G.
Presynaptic actions of endocannabinoids mediate alpha-MSH-induced inhibition of oxytocin cells. Am J Physiol Regul Integr Comp Physiol. 290(3):R577-84, 2006.

- Tobin DJ, Kauser S.
Hair melanocytes as neuro-endocrine sensors--pigments for our imagination. Mol Cell Endocrinol. 243(1-2):1-11, 2005.

- Zhang L, Li WH, Anthonavage M, Eisinger M.
Melanocortin-5 receptor: a marker of human sebocyte differentiation. Peptides. 27(2):413-20, 2006.

Clinical Investigation

- Cragnolini AB, Schioth HB, Scimonelli TN.
Anxiety-like behavior induced by IL-1beta is modulated by alpha-MSH through central melanocortin-4 receptors. Peptides. 2005 Nov 29.

- Dhillo WS, Gardiner JV, Castle L, Bewick GA, Smith KL, Meeran K, Todd JF, Ghatei MA, Bloom SR.
Agouti related protein (AgRP) is upregulated in Cushing's syndrome. Exp Clin Endocrinol Diabetes. 113(10):602-6, 2005.

- Lee YS, Challis BG, Thompson DA, Yeo GS, Keogh JM, Madonna ME, Wraight V, Sims M, Vatin V, Meyre D, Shield J, Burren C, Ibrahim Z, Cheetham T, Swift P, Blackwood A, Hung CC, Wareham NJ, Froguel P, Millhauser GL, O'Rahilly S, Farooqi IS.
A POMC variant implicates beta-melanocyte-stimulating hormone in the control of human energy balance. Cell Metab. 3(2):135-40, 2006.

- Newman EA, Chai BX, Zhang W, Li JY, Ammori JB, Mulholland MW.
Activation of the Melanocortin-4 Receptor Mobilizes Intracellular Free Calcium in Immortalized Hypothalamic Neurons. J Surg Res. 2006 Mar 30.

Others - Not classified

- Forslin Aronsson S, Spulber S, Popescu LM, Winblad B, Post C, Oprica M, Schultzberg M.
alpha-Melanocyte-stimulating hormone is neuroprotective in rat global cerebral ischemia. Neuropeptides. 40(1):65-75, 2006.

- Kasper RS, Shved N, Takahashi A, Reinecke M, Eppler E.
A systematic immunohistochemical survey of the distribution patterns of GH, prolactin, somatolactin, beta-TSH, beta-FSH, beta-LH, ACTH, and alpha-MSH in the adenohypophysis of Oreochromis niloticus, the Nile tilapia. Cell Tissue Res. 2006 Mar 22.

- Lee TH, Jawan B, Chou WY, Lu CN, Wu CL, Kuo HM, Concejero AM, Wang CH.
alpha-Melanocyte-stimulating hormone gene therapy reverses carbon tetrachloride induced liver fibrosis in mice. J Gene Med. 2006 Feb 28.

- Maaser C, Kannengiesser K, Specht C, Luegering A, Brzoska T, Luger TA, Domschke W, Kucharzik T.
Crucial role of the melanocortin receptor MC1R in experimental colitis. Gut. 2006 Mar 16.

- Martin NM, Smith KL, Bloom SR, Small CJ.
Interactions between the melanocortin system and the hypothalamo-pituitary-thyroid axis. Peptides. 27(2):333-9, 2006.

- Menyhert J, Wittmann G, Hrabovszky E, Keller E, Liposits Z, Fekete C.
Interconnection between orexigenic neuropeptide Y- and anorexigenic alpha-melanocyte stimulating hormone-synthesizing neuronal systems of the human hypothalamus. Brain Res. 1076(1):101-5, 2006.

- Reinke E, Fabry Z.
Breaking or making immunological privilege in the central nervous system: the regulation of immunity by neuropeptides. Immunol Lett. 104(1-2):102-9, 2006.

- Wang CH, Lee TH, Lu CN, Chou WY, Hung KS, Concejero AM, Jawan B.
Electroporative alpha-MSH gene transfer attenuates thioacetamide-induced murine hepatic fibrosis by MMP and TIMP modulation. Gene Ther. 2006 Mar 2.

4. Photobiology

(Dr. N. Smit)

The paper by Rhodes points out that the public messages emphasizing the role of UVR in tumour development has resulted in a general believe that melanoma is caused by UVR exposure. The author indicates that we do not know the exact contribution of UVR on melanoma and prevention strategies should not only be directed towards protection against UVR exposure. Skin awareness and e.g. total skin screening, especially for patients with high melanoma risk (historically and phenotypically) could have a more immediate positive impact on melanoma mortality. Familial melanoma, as reviewed by Pho et al, develops early and patients are prone to develop multiple primary tumours. Also for the familial melanomas the exact contribution of UVR is unclear. The familial melanoma could serve as an excellent model to improve the understanding of genotype-phenotype and environmental relationships in the pathogenesis of melanoma. Niendorf and Tsao also mention genetic determinants as modulators of melanoma risk that are probably more important than environmental (UVR) exposure. Another paper by Berwick and Wiggins also discusses the modest role of UVR with only a 1.7 fold risk for developing melanoma, thus focus is now on genotypic and phenotypic factors.
The paper by Wang et al is another example of a recent study on mutagen sensitivity that once more demonstrates the role of UV in NMSC but not in melanoma.
The idea of Rhodes about the general believe that melanoma is caused by UVR exposure is confirmed in the paper by Roussaki-Schulze et al. After they described that no significant relationship between melanoma and HPV infection could be demonstrated, UVR exposure is still considered as the main cause of melanoma in the region. Nevertheless five out of 28 patients were positive for HPV DNA (and 0 out of 6 controls). Although this was not significant it does not rule out a role for HPV in the positive cases. In the paper by Akgul et al the literature is reviewed on the role of HPV infection and UV in skin carcinogenis (mostly NMSC). In case of the non-melanoma skin cancers, especially the immunosupressed renal transplant patient have a markedly increased (~ 200 –fold) incidence of SCC, especially on sunexposed body sites. Unfortunately, the immunosuppressive role of UVR exposure is not discussed in this review. Several papers may give some more insight in the immunosuppressive mechanisms caused by UVR exposure.
In the paper by Hernandez-Pigeon et al it is shown that next to melanocytes also T-lymphocytes are targets for granzyme B and perforin dependent apoptosis. Expression of GrB and PFN is induced in keratinocytes by UV-B and influenced by redox signalling. Pedeux et al have studied the sensitivity of peripheral lymphocytes for UV-B as risk factor for melanoma. They show that the lymphocytes in melanoma patients are more sensitive to UV-B induced apoptosis. McGee et al aimed to shed some light on the link between UV-B irradiation in childhood and melanoma at later age. In mice they found that neonatal Langerhans cells (LC) were more susceptible to depletion by UV-B than adult LC. At maturity however an enhanced immune response was observed in the mice system. Fourtanier et al show the protective effects of different sunscreens on various skin cell types and skin equivalent model systems. In humans they found a depletion of LC after solar simulated radiation (SSR) and protection by sunscreens which was much better for the sunscreen with high UV-A protection factor (PF). Also for the delayed type hypersensitivity (DTH) response improved protection was obtained for the sunscreen with better UVA-PF. Results indicate an important role for UVA in immunosupression. Earlier melanocyte studies showed a higher sensitivity to DNA breaks than unpigmented cells (shown by the comet assay). Also for the melanocytes it was shown that better protection against the photooxidative damage was provided by the sunscreen with higher UVA-PF. Papers by Maresca et al and Haywood et al may give some explanation on the roles of melanin in the UVA induced photooxidation reactions. Maresca et al nicely demonstrated the effects of cysteinyl-DOPA melanin on UVA induced changes in the catalase electrophoretic properties indicating the free radical mediated protein oxidation in myeloid U937 cells. In a commentary in the same issue of the J. Invest. Dermatol. Wood and Schallreuter support the data on photooxidation of catalase and expand on them by clarifying the structural modelling of the enzyme and showing oxidation of the methionione and tryptophan residues.
Haywood et al used different sources of melanin to show the production of hydroxyl and hydroperoxyl radicals using DMPO as a spin trap. In the case of natural eumelanins one might question the purity of the eumelanin polymer and other compounds could interfere. In case of synthetic eumelanin however also superoxide was produced as a photooxidation product. This suggests that not only pheomelanin may act as a photosensitizer.

- Abdulla FR, Feldman SR, Williford PM, Krowchuk D, Kaur M.
  Tanning and skin cancer. Pediatr.Dermatol. 22:501-512, 2005.

- Agredano YZ, Chan JL, Kimball RC, Kimball A.
  Accessibility to air travel correlates strongly with increasing melanoma incidence. Melanoma Res. 16:77-81, 2006.

- Akgul B, Cooke JC, Storey A.
  HPV-associated skin disease. J.Pathol. 208:165-175, 2006.

- Baliga MS, Katiyar SK.
  Chemoprevention of photocarcinogenesis by selected dietary botanicals. Photochem.Photobiol.Sci. 5:243-253, 2006.

This review summarizes chemopreventive effects of some selected botanicals, such as apigenin, curcumin, grape seed proanthocyanidins, resveratrol, silymarin, and green tea polyphenols, against photocarcinogenesis in in vitro and in vivo systems.

- Berwick M, Wiggins C.
The current epidemiology of cutaneous malignant melanoma. Front Biosci. 11:1244-54:1244-1254, 2006.

- Elyassaki W, Wu S.
Lipid Rafts Mediate Ultraviolet Light-Induced FAS Aggregation in M624 Melanoma Cells. Photochem.Photobiol. 2006.
Based upon our results, we propose a novel mechanism by which UV induces apoptosis through a membrane lipid raft mediated signaling pathway

- Fourtanier A, Bernerd F, Bouillon C, Marrot L, Moyal D, Seite S.
Protection of skin biological targets by different types of sunscreens. Photodermatology Photoimmunology & Photomedicine 22:22-32, 2006.

- Ha L, Noonan FP, De Fabo EC, Merlino G.
Animal models of melanoma. J.Investig.Dermatol.Symp.Proc. 10:86-88, 2005.

- Haywood RM, Lee M, Linge C.
Synthetic melanin is a model for soluble natural eumelanin in UVA-photosensitised superoxide production. Journal of Photochemistry and Photobiology B-Biology 82:224-235, 2006.

- Hernandez-Pigeon H, Jean C, Charruyer A, Haure MJ, Titeux M, Tonasso L, Quillet-Mary A, Baudouin C, Charveron M, Laurent G.
Human keratinocytes acquire cellular cytotoxicity under UV-B irradiation: Implication of granzyme B and perforin. J.Biol.Chem. 2006**.**.
Furthermore under UV-irradiation, keratinocytes acquire a significant GrB and PFN dependent cytotoxicity, towards a variety of cellular targets including transformed T-lymphocytes, melanocytes and keratinocytes.

- Hung CF, Chiang HS, Lo HM, Jian JS, Wu WB.
E-cadherin and its downstream catenins are proteolytically cleaved in human HaCaT keratinocytes exposed to UVB. Exp.Dermatol. 15:315-321, 2006.

- Kulesz-Martin M, Lagowski J, Fei S, Pelz C, Sears R, Powell MB, Halaban R, Johnson J.
Melanocyte and keratinocyte carcinogenesis: p53 family protein activities and intersecting mRNA expression profiles. J.Investig.Dermatol.Symp.Proc. 10:142-152, 2005.
Clonal lineage mouse models representing early through late cancer progression stages may inform the focus on early, potentially causal events from microarray studies of human cancers, facilitating prognosis and molecular therapy

- Leone G, Pacifico A, Iacovelli P, Vidolin AP, Picardo M.
Tacalcitol and narrow-band phototherapy in patients with vitiligo. Clinical and Experimental Dermatology 31:200-205, 2006.

- Li LH, Wu LJ, Tashiro SI, Onodera S, Uchiumi F, Ikejima T.
The roles of Akt and MAPK family members in silymarin's protection against UV-induced A375-S2 cell apoptosis. Int.Immunopharmacol. 6:190-197, 2006.

- Maresca V, Flori E, Briganti S, Camera E, Cario-Andre M, Taieb A, Picardo M.
UVA-induced modification of catalase charge properties in the epidermis is correlated with the skin phototype. J.Invest Dermatol. 126:182-190, 2006.

- McGee HM, Scott DK, Woods GM.
Neonatal exposure to UV-B radiation leads to a large reduction in Langerhans cell density, but by maturity, there is an enhanced ability of dendritic cells to stimulate T cells. Immunol.Cell Biol. 2006.

- Middelkamp-Hup MA, Park HY, Lee J, Gilchrest BA, Gonzalez S.
Detection of UV-induced pigmentary and epidermal changes over time using in vivo reflectance confocal microscopy. J.Invest Dermatol.126:402-407, 2006.

- Niendorf KB, Tsao H.
Cutaneous melanoma: family screening and genetic testing. Dermatol.Ther. 19:1-8, 2006.

- Pedeux R, Sales F, Pourchet J, Kallassy M, Fayolle C, Boniol M, Severi G, Ghanem G, Nakazawa HN, Autier P, Dore JF.
Ultraviolet B sensitivity of peripheral lymphocytes as an independent risk factor for cutaneous melanoma. Eur.J.Cancer. 42:212-215, 2006.

- Pho L, Grossman D, Leachman SA.
Melanoma genetics: a review of genetic factors and clinical phenotypes in familial melanoma. Curr.Opin.Oncol. 18:173-179, 2006.

- Rhodes AR.
Cutaneous melanoma and intervention strategies to reduce tumor-related mortality: what we know, what we don't know, and what we think we know that isn't so. Dermatol.Ther. 19:50-69, 2006.

- Roussaki-Schulze AV, Kouskoukis C, Rammos C, Rallis E, Kontos F, Zafiriou E, Gross G.
Identification of human papillomavirus DNA in melanoma biopsy specimens of Greek population. Int.J.Clin.Pharmacol.Res. 25:145-150, 2005.

- Takata M, Saida T.
Early cancers of the skin: clinical, histopathological, and molecular characteristics. Int.J.Clin.Oncol. 10:391-397, 2005.
Such precursor clones may be induced at a rather young age, and their number and size increase with accumulating carcinogenic stimuli. If these lesions acquire additional mutations, they could progress to clinically visible lesions of in situ carcinoma.

- Taylor SC.
Enhancing the care and treatment of skin of color, part 2: understanding skin physiology. Cutis 76:302-306, 2005.

- Wang LE, Xiong P, Strom SS, Goldberg LH, Lee JE, Ross MI, Mansfield PF, Gershenwald JE, Prieto VG, Cormier JN, Duvic M, Clayman GL, Weber RS, Lippman SM, Amos CI, Spitz MR, Wei Q.
In vitro sensitivity to ultraviolet B light and skin cancer risk: a case-control analysis. J.Natl.Cancer Inst. 97:1822-1831, 2005.

5. Neuromelanins

(Prof. M. d'Ischia)

Literature on neuromelanin in the first months of 2006 comprises two reviews and two research papers. One review by Keller (2006) addresses the molecular and cellular processes that occur during brain aging and during age-related disorders of the central nervous system, dealing only peripherally with neuromelanin. Another review, provided by Tribl et al. (2006) is focused on the analysis of neuromelanin granules and their function as Fe accumulator in the human brain.
Kim et al. (2006) investigated age dependent changes in the dopaminergic neurones in mice comparing 7- and 50-wk-old animals. They demonstrated dramatic increases in tyrosine hydroxylase (TH) immunodensity as early as middle age along with an increase in the number of neuromelanin-containing neurones, volume of neuromelanin per cell, and number of degenerating neurones.
Fedorow et al. (2006) studied the age-related development and regulation of neuromelanin within dopamine neurons by examining the ventral substantia nigra neurons from 29 people spanning the ages of 24 wk to 95 years old. Three developmental phases were demonstrated, associated with the appearance of the pigment (3 years of age), an increase in the number of pigment granules and pigment granule coloration until 20 years of age, followed by a period of darkening without apparent growth in pigment volume. A regulatory mechanism of neuromelanin production and turnover, possibly through enzymic processes, was suggested, but verification of this suggestion awaits a more in-depth understanding of certain critical aspects of neuromelanin biogenesis, roles and functions.

- Fedorow H., Halliday G. M., Rickert C. H., Gerlach M., Riederer P., Double K. L.
  Evidence for specific phases in the development of human neuromelanin. Neurobiology of Aging. 27(3), 506-512, 2006.
  Abstract**:** Neuromelanin is a dark-colored pigment which forms in the dopamine neurons of the human midbrain. The age-related development and regulation of neuromelanin within these dopamine neurons has not been previously described. Optical d. and area measurements of unstained neuromelanin in ventral substantia nigra neurons from 29 people spanning the ages of 24 wk to 95 years old, demonstrated three developmental phases. Neuromelanin was not present at birth and initiation of pigmentation began at approx. 3 years of age, followed by a period of increasing pigment granule no. and increasing pigment granule coloration until age 20. In middle and later life the color of the pigment granules continued to darken but was not assocd. with any substantial growth in pigment vol. The identification of three phases and changes in the rate of neuromelanin prodn. over time suggests the regulation of neuromelanin prodn. and turnover, possibly through enzymic processes.

- Keller Jeffrey N.
  Age-related neuropathology, cognitive decline, and Alzheimer's disease. Ageing Research Reviews, 5(1), 1-13, 2006.
  Abstract **:** In the last 20 years, there have been tremendous strides made in the understanding of the mol. and cellular processes that occur during brain aging, as well as our understanding of age-related disorders of the central nervous system (CNS). Aging is assocd. with a decline in cognitive performance, and is the biggest risk factor for the development of Alzheimer's disease (AD), although the underlying basis for both of these observations is poorly defined. Both normal aging and AD are assocd. with overlapping and increased levels of pathol. Numerous reports have now linked elevations in pathol. as potential mediators of cognitive decline in the elderly, with most studies focusing on the role of AD-related pathol. However, it is important to point out that there are numerous other pathol. features obsd. in the aging brain including corpora amylacea, argyrophilic grains, neuromelanin, and lipofuscin. In this review, I discuss the decreased cognitive performance obsd. during normal aging, the potential for pathol. to alter neuronal function and neuronal viability during normal brain aging, and the potential for common pathologies to either inhibit or promote the development of age-related disorders such as AD.

- Kim Sung Tae, Choi Ji Hyun, Kim DongHou, Hwang Onyou.
  Increases in TH immunoreactivity, neuromelanin and degeneration in the substantia nigra of middle aged mice. Neuroscience Letters 396(3), 263-268, 2006.
  Abstract**:** The dopaminergic (DArgic) neurons in the substantia nigra (SN) are particularly vulnerable to oxidative stress and during aging. The present study was undertaken in order to det. whether aging is assocd. with changes in the DA synthesizing enzyme tyrosine hydroxylase (TH) as early as middle age by comparing 7- and 50-wk-old mice. Quant.anal performed by measuring the d. of TH-immunopos. neurons, revealed that in the older animals, the no. of DArgic neurons was decreased by 10% while TH immunodensity was 24 3% higher compared to the younger animals. Based on Masson-Fontana staining for neuromelanin (NM), the no.of NM-contg. neurons in the SN and the vol. of NM per NM-pos. neurons in the older animals were 5- and 11.6 0.1-fold higher, resp. The silver stain-pos. fibers, indicative of degeneration, were higher in the SN and striatum of the older animals, with the optical d. 3.3 0.1- and 5.4 0.2-fold of the younger animals. The present study demonstrates that aging is assocd. with changes in the DA synthesizing enzyme TH as early as middle age and that this is assocd. with dramatic increases in the no. of NM-contg. neurons, vol. of NM per cell, and degeneration.

- Tribl Florian, Riederer Peter; et al.

Neuromelanin granula. Bioforum 28(11), 28-30, 2005.

Abstract**:** A review is given on the anal. of neuromelanin granules by sub-cellular proteome anal. The biol. of neuromelanin granules and their function as Fe accumulator in the human brain are described. Pathways of biogenesis involving endosomal compartments are discussed.

6. Genetics, molecular and developmental biology

(Dr. F. Beermann)

Transgenic models:

(1) The past year saw the publication of 3 novel transgenic models for mouse melanoma. In contrast to previous models, occuring melanoma were pigmented and metastasized to lymph nodes. Ackermann et al. (Cancer Research. 2005) used melanocyte-specific expression of oncogenic N-ras^{Q61K} in transgenic mice which were deficient for tumor suppressors p16^{INK4a} and p19^{ARF}. Hacker et al., (Cancer Research. 2006, see below) used mice expressing mutant Ha-ras affected at another melanoma-prone locus in human, CDK4. A very complicated and sophisticated approach was chosen by Huijbers et al., (Cancer Research. 2006, see below), where Ha-ras expression and deletion of Ink4a/Arf was inducible and accompanied by expression of a defined tumor antigen.

(2) Lavado et al. (Journal of Neurochemistry 2006, see below) have provided evidence that it is not the presence of pigment which is required for proper retinal development and correct routing of hemispheric pathways. Following transgenic expression of tyrosine hydroxylase in the retinal pigment epithelium of albino mice, retinal abnormalities and visual function were completely restored.

- Goding C, Meyskens FL, Jr.
Microphthalmic-associated transcription factor integrates melanocyte biology and melanoma progression. Clin Cancer Res 12(4):1069-1073, 2006.

- Grichnik JM, Burch JA, Schulteis RD, Shan S, Liu J, Darrow TL, Vervaert CE, Seigler HF.
Melanoma, a tumor based on a mutant stem cell? J Invest Dermatol 126(1):142-153, 2006.

- Haass NK, Herlyn M.
Normal human melanocyte homeostasis as a paradigm for understanding melanoma. J Investig Dermatol Symp Proc 10(2):153-163, 2005.

- Hacker E, Muller HK, Irwin N, Gabrielli B, Lincoln D, Pavey S, Powell MB, Malumbres M, Barbacid M, Hayward N, Walker G.
Spontaneous and UV radiation-induced multiple metastatic melanomas in Cdk4R24C/R24C/TPras mice. Cancer Res 66(6):2946-2952, 2006.
Shortened abstract: Here, we report that activated Cdk4 cooperates with activated Hras to enhance susceptibility to melanoma in mice. Whereas UVR treatment failed to induce melanomas in Cdk4(R24C/R24C) mice, it greatly increased the penetrance and decreased the age of onset of melanoma development in Cdk4(R24C/R24C)/TPras animals compared with TPras alone. This increased penetrance was dependent on the threshold of Cdk4 activation as Cdk4(R24C/+)/TPras animals did not show an increase in UVR-induced melanoma penetrance compared with TPras alone. In addition, Cdk4(R24C/R24C)/TPras mice invariably developed multiple lesions, which occurred rarely in TPras mice. These results indicate that germ-line defects abrogating the pRb pathway may enhance UVR-induced melanoma. TPras and Cdk4(R24C/R24C)/TPras tumors were comparable histopathologically but the latter were larger and more aggressive and cultured cells derived from such melanomas were also larger and had higher levels of nuclear atypia. Moreover, the melanomas in Cdk4(R24C/R24C)/TPras mice, but not in TPras mice, readily metastasized to regional lymph nodes. Thus, it seems that in the mouse, Hras activation initiates UVR-induced melanoma development whereas the cell cycle defect introduced by mutant Cdk4 contributes to tumor progression, producing more aggressive, metastatic tumors.

- Hakami RM, Hou L, Baxter LL, Loftus SK, Southard-Smith EM, Incao A, Cheng J, Pavan WJ.
Genetic evidence does not support direct regulation of EDNRB by SOX10 in migratory neural crest and the melanocyte lineage. Mech Dev 123(2):124-134, 2006.

- Hattab FN, Amin WM.
Papillon-Lefevre syndrome with albinism: a review of the literature and report of 2 brothers. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 100(6):709-716, 2005.

- Hedan B, Corre S, Hitte C, Dreano S, Vilboux T, Derrien T, Denis B, Galibert F, Galibert MD, Andre C.
Coat colour in dogs: identification of the Merle locus in the Australian shepherd breed. BMC Vet Res 2(1):9, 2006.

- Hoogduijn MJ, Hitchcock IS, Smit NP, Gillbro JM, Schallreuter KU, Genever PG.
Glutamate receptors on human melanocytes regulate the expression of MiTF. Pigment Cell Res 19(1):58-67, 2006.

- Huijbers IJ, Krimpenfort P, Chomez P, van der Valk MA, Song JY, Inderberg-Suso EM, Schmitt-Verhulst AM, Berns A, Van den Eynde BJ.
An inducible mouse model of melanoma expressing a defined tumor antigen. Cancer Res 66(6):3278-3286, 2006.

Shortened abstract: To create a faithful preclinical model for cancer immunotherapy, we generated a transgenic mouse strain developing autologous melanomas expressing a defined tumor antigen recognized by T cells. We chose the antigen encoded by P1A, a well-characterized murine cancer germ line gene. To transform melanocytes, we aimed at simultaneously activating the Ras pathway and inactivating tumor suppressor Ink4a/Arf, thereby reproducing two genetic events frequently observed in human melanoma. The melanomas are induced by s.c. injection of 4-OH-tamoxifen (OHT). By activating a CreER recombinase expressed from a melanocyte-specific promoter, this treatment induces the loss of the conditional Ink4a/Arf gene in melanocytes. Because the CreER gene itself is also flanked by loxP sites, the activation of CreER also induces the deletion of its own coding sequence and thereby allows melanocyte-specific expression of genes H-ras and P1A, which are located downstream on the same transgene. All melanomas induced in those mice with OHT show activation of the Ras pathway and deletion of gene Ink4a/Arf. In addition, these melanomas express P1A and are recognized by P1A-specific T lymphocytes. This model will allow to characterize the interactions between the immune system and naturally occurring tumors and thereby to optimize immunotherapy approaches targeting a defined tumor antigen.

- James MR, Roth RB, Shi MM, Kammerer S, Nelson MR, Stark MS, Dumenil T, Montgomery GW, Hayward NK, Martin NG, Braun A, Duffy DL.
BRAF polymorphisms and risk of melanocytic neoplasia. J Invest Dermatol 125(6):1252-1258, 2005.

- Koyanagi K, O'Day SJ, Gonzalez R, Lewis K, Robinson WA, Amatruda TT, Kuo C, Wang HJ, Milford R, Morton DL, Hoon DS.
Microphthalmia transcription factor as a molecular marker for circulating tumor cell detection in blood of melanoma patients. Clin Cancer Res 12(4):1137-1143, 2006.

- Kulesa PM, Kasemeier-Kulesa JC, Teddy JM, Margaryan NV, Seftor EA, Seftor RE, Hendrix MJ.
Reprogramming metastatic melanoma cells to assume a neural crest cell-like phenotype in an embryonic microenvironment. Proc Natl Acad Sci U S A 103(10):3752-3757, 2006.
Summary: Using an embryonic chick model, the authors test the possibility of reverting the metastatic melanoma phenotype to its cell type of origin, the neural-crest-derived melanocyte. The results demonstrate the ability of adult human metastatic melanoma cells to respond to chick embryonic environmental cues, a subset of which may undergo a reprogramming of their metastatic phenotype

- Lamason RL, Mohideen MA, Mest JR, Wong AC, Norton HL, Aros MC, Jurynec MJ, Mao X, Humphreville VR, Humbert JE, Sinha S, Moore JL, Jagadeeswaran P, Zhao W, Ning G, Makalowska I, McKeigue PM, O'Donnell D, Kittles R, Parra EJ, Mangini NJ, Grunwald DJ, Shriver MD, Canfield VA, Cheng KC.
SLC24A5, a putative cation exchanger, affects pigmentation in zebrafish and humans. Science 310(5755):1782-1786, 2005.
Abstract: Lighter variations of pigmentation in humans are associated with diminished number, size, and density of melanosomes, the pigmented organelles of melanocytes. Here we show that zebrafish golden mutants share these melanosomal changes and that golden encodes a putative cation exchanger slc24a5 (nckx5) that localizes to an intracellular membrane, likely the melanosome or its precursor. The human ortholog is highly similar in sequence and functional in zebrafish. The evolutionarily conserved ancestral allele of a human coding polymorphism predominates in African and East Asian populations. In contrast, the variant allele is nearly fixed in European populations, is associated with a substantial reduction in regional heterozygosity, and correlates with lighter skin pigmentation in admixed populations, suggesting a key role for the SLC24A5 gene in human pigmentation.

- Lavado A, Jeffery G, Tovar V, de la Villa P, Montoliu L.
Ectopic expression of tyrosine hydroxylase in the pigmented epithelium rescues the retinal abnormalities and visual function common in albinos in the absence of melanin. J Neurochem 96(4):1201-1211., 2006.
Abstract: Albino mammals have profound retinal abnormalities, including photoreceptor deficits and misrouted hemispheric pathways into the brain, demonstrating that melanin or its precursors are required for normal retinal development. Tyrosinase, the primary enzyme in melanin synthesis commonly mutated in albinism, oxidizes l-tyrosine to l-dopaquinone using l-3,4-dihydroxyphenylalanine (L-DOPA) as an intermediate product. L-DOPA is known to signal cell cycle exit during retinal development and plays an important role in the regulation of retinal development. Here, we have mimicked L-DOPA production by ectopically expressing tyrosine hydroxylase in mouse albino retinal pigment epithelium cells. Tyrosine hydroxylase can only oxidize l-tyrosine to L-DOPA without further progression towards melanin. The resulting transgenic animals remain phenotypically albino, but their visual abnormalities are corrected, with normal photoreceptor numbers and hemispheric pathways and improved visual function, assessed by an increase of spatial acuity. Our results demonstrate definitively that only early melanin precursors, L-DOPA or its metabolic derivatives, are vital in the appropriate development of mammalian retinae. They further highlight the value of substituting independent but biochemically related enzymes to overcome developmental abnormalities.

- Li W, He M, Zhou H, Bourne JW, Liang P.
Mutational data integration in gene-oriented files of the Hermansky-Pudlak Syndrome database. Hum Mutat 20 Epub, 2006.

- Mak SS, Moriyama M, Nishioka E, Osawa M, Nishikawa S.
Indispensable role of Bcl2 in the development of the melanocyte stem cell. Dev Biol 291(1):144-153, 2006.
Summary: The results demonstrate that Bcl2 has a general role in melanoblast and melanocyte survival and is essential for the emergence of melanocyte stem cells. Moreover, the results indicate that the first wave of melanocyte stem cells that provide hair pigmentation is derived directly from epidermal melanoblasts bypassing melanocyte stem cells. Furthermore, a Bcl2-independent mechanism of action of SCF in the melanocyte lineage is revealed as SCF c-Kit signaling is functional in the absence of Bcl2.

- McGill GG, Haq R, Nishimura EK, Fisher DE.
c-met expression is regulated by mitf in the melanocyte lineage. J Biol Chem Epub, 2006.

- Meierjohann S, Wende E, Kraiss A, Wellbrock C, Schartl M.
The oncogenic epidermal growth factor receptor variant Xiphophorus melanoma receptor kinase induces motility in melanocytes by modulation of focal adhesions. Cancer Res 66(6):3145-3152, 2006.

- Miwa M, Inoue-Murayama M, Kobayashi N, Kayang BB, Mizutani M, Takahashi H, Ito S.
Mapping of panda plumage color locus on the microsatellite linkage map of the Japanese quail. BMC Genet 7, 2006.

- Morgan NV, Pasha S, Johnson CA, Ainsworth JR, Eady RA, Dawood B, McKeown C, Trembath RC, Wilde J, Watson SP, Maher ER.
A germline mutation in BLOC1S3/reduced pigmentation causes a novel variant of Hermansky-Pudlak syndrome (HPS8). Am J Hum Genet 78(1):160-166, 2006.

- Murisier F, Beermann F.
Genetics of pigment cells: lessons from the tyrosinase gene family. Histol Histopathol 21(5):567-578, 2006.

- Nelson MA, Reynolds SH, Rao UN, Goulet AC, Feng Y, Beas A, Honchak B, Averill J, Lowry DT, Senft JR, Jefferson AM, Johnson RC, Sargent LM.
Increased Gene Copy Number of The Transcription Factor E2F1 in Malignant Melanoma. Cancer Biol Ther 5 Epub, 2006.

- Nickoloff BJ, Hendrix MJ, Pollock PM, Trent JM, Miele L, Qin JZ.
Notch and NOXA-related pathways in melanoma cells. J Investig Dermatol Symp Proc 10(2):95-104, 2005.

- Osawa M, Egawa G, Mak SS, Moriyama M, Freter R, Yonetani S, Beermann F, Nishikawa S.
Molecular characterization of melanocyte stem cells in their niche. Development 132(24):5589-5599, 2005.
Shortened abstract: Here, we present molecular makers that can distinguish MSCs from other melanocyte (MC) subsets in the HF. We also describe a simple and robust method that allows gene expression profiling in individual SCs. After isolating individual MSCs from transgenic mice in which the MCs are marked by green fluorescence protein (GFP), we performed single-cell transcript analysis to obtain the molecular signature of individual MSCs in the niche. The data suggest the existence of a mechanism that induces the downregulation of various key molecules for MC proliferation or differentiation in MSCs located in the niche. By integrating these data, we propose that the niche is an environment that insulates SCs from various activating stimuli and maintains them in a quiescent state.

- Protas ME, Hersey C, Kochanek D, Zhou Y, Wilkens H, Jeffery WR, Zon LI, Borowsky R, Tabin CJ.
Genetic analysis of cavefish reveals molecular convergence in the evolution of albinism. Nat Genet 38(1):107-111, 2006.
Comment: A study on evolution: Separate populations of cavefish developed albinism independently, but through a similar mutation event, targeting the same gene (OCA2).

- Roberts DW, Newton RA, Beaumont KA, Helen Leonard J, Sturm RA.
Quantitative analysis of MC1R gene expression in human skin cell cultures. Pigment Cell Res 19(1):76-89, 2006.

- Runkel F, Bussow H, Seburn KL, Cox GA, Ward DM, Kaplan J, Franz T.
Grey, a novel mutation in the murine Lyst gene, causes the beige phenotype by skipping of exon 25. Mamm Genome 17(3):203-210, 2006.

- Santiago Borrero PJ, Rodriguez-Perez Y, Renta JY, Izquierdo NJ, Del Fierro L, Munoz D, Molina NL, Ramirez S, Pagan-Mercado G, Ortiz I, Rivera-Caragol E, Spritz RA, Cadilla CL.
Genetic testing for oculocutaneous albinism type 1 and 2 and Hermansky-Pudlak syndrome type 1 and 3 mutations in Puerto Rico. J Invest Dermatol 126(1):85-90, 2006.

- Sharov A, Tobin DJ, Sharova TY, Atoyan R, Botchkarev VA.
Changes in different melanocyte populations during hair follicle involution (catagen). J Invest Dermatol 125(6):1259-1267, 2005.

- Stewart RA, Arduini BL, Berghmans S, George RE, Kanki JP, Henion PD, Look AT.
Zebrafish foxd3 is selectively required for neural crest specification, migration and survival. Dev Biol 21 Epub, 2006.

- Tosaki H, Kunisada T, Motohashi T, Aoki H, Yoshida H, Kitajima Y.
Mice Transgenic for Kit(V620A): Recapitulation of Piebaldism but not Progressive Depigmentation Seen in Humans with this Mutation. J Invest Dermatol 2 Epub, 2006.

- Verheij JB, Sival DA, van der Hoeven JH, Vos YJ, Meiners LC, Brouwer OF, van Essen AJ.
Shah-Waardenburg syndrome and PCWH associated with SOX10 mutations: A case report and review of the literature. Eur J Paediatr Neurol 24 Epub, 2006.

- Vetrini F, Tammaro R, Bondanza S, Surace EM, Auricchio A, De Luca M, Ballabio A, Marigo V.
Aberrant splicing in the ocular albinism type 1 gene (OA1/GPR143) is corrected in vitro by morpholino antisense oligonucleotides. Hum Mutat 20 Epub, 2006.

- Voigt J, Papalopulu N.
A dominant-negative form of the E3 ubiquitin ligase Cullin-1 disrupts the correct allocation of cell fate in the neural crest lineage. Development 133(3):559-568, 2006.
Shortened abstract: We describe the identification of Xenopus Cullin-1, an E3 ubiquitin ligase, and show that blocking the function of endogenous Cullin-1 leads to pleiotropic defects in development. Notably, there is an increased allocation of cells to a neural crest fate and within this lineage, an increase in melanocytes at the expense of cranial ganglia neurons. Most of the observed effects can be attributed to stabilisation of beta-catenin, a known target of Cullin-1-mediated degradation from other systems. Indeed, we show that blocking the function of Cullin-1 leads to a decrease in ubiquitinated beta-catenin and an increase in total beta-catenin. Our results show that Cullin-1-mediated protein degradation plays an essential role in the correct allocation of neural crest fates during embryogenesis.

- Wang R, Tang P, Wang P, Boissy RE, Zheng H.
Regulation of tyrosinase trafficking and processing by presenilins: partial loss of function by familial Alzheimer's disease mutation. Proc Natl Acad Sci U S A 103(2):353-358, 2006.
Abstract: Presenilins (PS) are required for gamma-secretase cleavage of multiple type I membrane proteins including the amyloid precursor protein and Notch and also have been implicated in regulating intracellular protein trafficking and turnover. Using genetic and pharmacological approaches, we reveal here a unique function of PS in the pigmentation of retinal pigment epithelium and epidermal melanocytes. PS deficiency leads to aberrant accumulation of tyrosinase (Tyr)-containing 50-nm post-Golgi vesicles that are normally destined to melanosomes. This trafficking is gamma-secretase-dependent, and abnormal localization of Tyr in the absence of PS is accompanied by the simultaneous accumulation of its C-terminal fragment. Furthermore, we show that the PS1M146V familial Alzheimer's disease mutation exhibits a partial loss-of-function in pigment synthesis. Our results identify Tyr and related proteins as physiological substrates of PS and link gamma-secretase activity with intracellular protein transport.

- Wei ML.
Hermansky-Pudlak syndrome: a disease of protein trafficking and organelle function. Pigment Cell Res 19(1):19-42., 2006.

- Wolnicka-Glubisz A, Noonan FP.
Neonatal susceptibility to UV induced cutaneous malignant melanoma in a mouse model. Photochem Photobiol Sci 5(2):254-260, 2006.

- Yajima I, Belloir E, Bourgeois Y, Kumasaka M, Delmas V, Larue L.
Spatiotemporal gene control by the Cre-ERT2 system in melanocytes. Genesis 44(1):34-43, 2006.
Comment: A transgenic mouse strain allowing for inducible gene knockouts in melanocytes
(see Huijbers et al. (2006) for a related approach).

- Yamada T, Ohtani S, Sakurai T, Tsuji T, Kunieda T, Yanagisawa M.
Reduced expression of endothelin receptor type B gene in piebald mice caused by an insertion of a retroposon-like element in intron 1. J Biol Chem 24 Epub, 2006.

7. Tyrosinase, TRPs, other enzymes

(Prof. J.C. Garcia-Borron)

- Ando H, Wen ZM, Kim HY, Valencia JC, Costin GE, Watabe H, Yasumoto K, Niki Y, Kondoh H, Ichihashi M, Hearing VJ.
  Intracellular composition of fatty acid affects the processing and function of tyrosinase through the ubiquitin-proteasome pathway. Biochem J. 394(Pt 1):43-50, 2006.
  Proteasomes are multicatalytic proteinase complexes within cells that selectively degrade ubiquitinated proteins. We have recently demonstrated that fatty acids, major components of cell membranes, are able to regulate the proteasomal degradation of tyrosinase, a critical enzyme required for melanin biosynthesis, in contrasting manners by relative increases or decreases in the ubiquitinated tyrosinase. In the present study, we show that altering the intracellular composition of fatty acids affects the post-Golgi degradation of tyrosinase. Incubation with linoleic acid (C18:2) dramatically changed the fatty acid composition of cultured B16 melanoma cells, i.e. the remarkable increase in polyunsaturated fatty acids such as linoleic acid and arachidonic acid (C20:4) was compensated by the decrease in monounsaturated fatty acids such as oleic acid (C18:1) and palmitoleic acid (C16:1), with little effect on the proportion of saturated to unsaturated fatty acid. When the composition of intracellular fatty acids was altered, tyrosinase was rapidly processed to the Golgi apparatus from the ER (endoplasmic reticulum) and the degradation of tyrosinase was increased after its maturation in the Golgi. Retention of tyrosinase in the ER was observed when cells were treated with linoleic acid in the presence of proteasome inhibitors, explaining why melanin synthesis was decreased in cells treated with linoleic acid and a proteasome inhibitor despite the abrogation of tyrosinase degradation. These results suggest that the intracellular composition of fatty acid affects the processing and function of tyrosinase in connection with the ubiquitin-proteasome pathway and suggest that this might be a common physiological approach to regulate protein degradation.

- Bonfigli A, Zarivi O, Colafarina S, Cimini AM, Ragnelli AM, Aimola P, Natali P, Ceru M, Amicarelli F, Miranda M.
  Human glioblastoma ADF cells express tyrosinase, L-tyrosine hydroxylase and melanosomes and are sensitive to L-tyrosine and phenylthiourea. J Cell Physiol. 2006 Jan 30; [Epub ahead of print]
  Melanocytes and neuroblasts share the property of transforming L-tyrosine through two distinct metabolic pathways leading to melanogenesis and catecholamine synthesis, respectively. While tyrosinase (TYR) activity has been shown to be expressed by neuroblastoma it remains to be established as to whether also glioblastomas cells are endowed with this property. We have addressed this issue using the human continuous glioblastoma cell line ADF. We demonstrated that these cells possess tyrosinase as well as L-tyrosine hydroxylase (TH) activity and synthesize melanosomes. Because the two pathways are potentially cyto-genotoxic due to production of quinones, semiquinones, and reactive oxygen species (ROS), we have also investigated the expression of the peroxisomal proliferators activated receptor alpha (PPARalpha) and nuclear factor-kB (NFkB) transcription factor as well the effect of L-tyrosine concentration on cell survival. We report that L-tyrosine down-regulates PPARalpha expression in ADF cells but not neuroblastoma and that this aminoacid and phenylthiourea (PTU) induces apoptosis in glioblastoma and neuroblastoma.

- Borovansky J, Edge R, Land E, Navaratnam S, Pavel S, Ramsden CA, Riley PA, Smit NP.
  Mechanistic studies of melanogenesis: the influence of N-substitution on dopamine quinone cyclization. Pigment Cell Res. 19(2):170-8, 2006.
  The influence of side-chain structure on the mode of reaction of ortho-quinone amines has been investigated with a view, ultimately, to developing potential methods of therapeutic intervention by manipulating the early stages of melanogenesis. Four N-substituted dopamine derivatives have been prepared and quinone formation studied using pulse radiolysis and tyrosinase-oximetry. Ortho-quinones with an amide or urea side chain were relatively stable, although evidence for slow formation of isomeric para-quinomethanes was observed. A thiourea derivative cyclized fairly rapidly (k = 1.7/s) to a product containing a seven-membered ring, whereas a related amidine gave more rapidly (k approximately 2.5 x 10(2)/s) a stable spirocyclic product. The results suggest that cyclization of amides, ureas and carbamates (NHCO-X; X = R, NHR or OR) does not occur and is not, therefore, a viable approach to the formation of tyrosinase-activated antimelanoma prodrugs. It is also concluded that for N-acetyldopamine spontaneous ortho-quinone to para-quinomethane isomerization is slow.

- Cardinali G, Ceccarelli S, Kovacs D, Aspite N, Lotti LV, Torrisi MR, Picardo M.
  Keratinocyte growth factor promotes melanosome transfer to keratinocytes. J Invest Dermatol. 125(6):1190-9, 2005.
  Melanogenesis and melanosome transfer from the melanocytes to the neighboring keratinocytes are induced by ultraviolet radiation and modulated by autocrine and paracrine factors. Keratinocyte growth factor (KGF/fibroblast growth factor (FGF)7) is a paracrine mediator of human keratinocyte growth and differentiation. We evaluated the influence of KGF on melanosome transfer in co-cultures of keratinocytes and melanocytes. Immunofluorescence analysis using anti-tyrosinase and anti-human cytokeratin antibodies, phagocytic assays using fluorescent latex beads, and ultrastructural analysis indicated that KGF is able to induce melanosome transfer acting only on the recipient keratinocytes and as a consequence of a general role of KGF in the promotion of the phagocytic process. Inhibition of

proteinase-activated receptor-2, to block the Rho-dependent phagocytic pathway, or of the Src family tyrosine kinases, to inhibit the Rac-dependent pathway, showed that KGF promotes phagocytosis through both mechanisms. Increased expression of the KGF receptor (KGFR) on the keratinocytes by transfection led to increased phagocytosis of latex beads following KGF treatment, suggesting that the KGF effect is directly mediated by KGFR expression and activation. Moreover, confocal microscopic analysis revealed that KGFR localize in phagosomes during KGF-induced phagocytosis, suggesting a direct role of the receptor in regulating both the early steps of uptake and the intracellular traffic of the phagosomes.

- Claus H, Decker H.
Bacterial tyrosinases. Syst Appl Microbiol. 29(1):3-14, 2006.
Tyrosinases are nearly ubiquitously distributed in all domains of life. They are essential for pigmentation and are important factors in wound healing and primary immune response. Their active site is characterized by a pair of antiferromagnetically coupled copper ions, CuA and CuB, which are coordinated by six histidine residues. Such a "type 3 copper centre" is the common feature of tyrosinases, catecholoxidases and haemocycanins. It is also one of several other copper types found in the multi-copper oxidases (ascorbate oxidase, laccase). The copper pair of tyrosinases binds one molecule of atmospheric oxygen to catalyse two different kinds of enzymatic reactions: (1) the ortho-hydroxylation of monophenols (cresolase activity) and (2) the oxidation of o-diphenols to o-diquinones (catecholase activity). The best-known function is the formation of melanins from L-tyrosine via L-dihydroxyphenylalanine (L-dopa). The complicated hydroxylation mechanism at the active centre is still not completely understood, because nothing is known about their tertiary structure. One main reason for this deficit is that hitherto tyrosinases from eukaryotic sources could not be isolated in sufficient quantities and purities for detailed structural studies. This is not the case for prokaryotic tyrosinases from different Streptomyces species, having been intensively characterized genetically and spectroscopically for decades. The Streptomyces tyrosinases are non-modified monomeric proteins with a low molecular mass of ca. 30kDa. They are secreted to the surrounding medium, where they are involved in extracellular melanin production. In the species Streptomyces, the tyrosinase gene is part of the melC operon. Next to the tyrosinase gene (melC2), this operon contains an additional ORF called melC1, which is essential for the correct expression of the enzyme. This review summarizes the present knowledge of bacterial tyrosinases, which are promising models in order to get more insights in structure, enzymatic reactions and functions of "type 3 copper" proteins in general.

- Fowler DM, Koulov AV, Alory-Jost C, Marks MS, Balch WE, Kelly JW.
Functional amyloid formation within mammalian tissue. PLoS Biol. 4(1):e6, 2006.
Amyloid is a generally insoluble, fibrous cross-beta sheet protein aggregate. The process of amyloidogenesis is associated with a variety of neurodegenerative diseases including Alzheimer, Parkinson, and Huntington disease. We report the discovery of an unprecedented functional mammalian amyloid structure generated by the protein Pmel17. This discovery demonstrates that amyloid is a fundamental nonpathological protein fold utilized by organisms from bacteria to humans. We have found that Pmel17 amyloid templates and accelerates the covalent polymerization of reactive small molecules into melanin-a critically important biopolymer that protects against a broad range of cytotoxic insults including UV and oxidative damage. Pmel17 amyloid also appears to play a role in mitigating the toxicity associated with melanin formation by sequestering and minimizing diffusion of highly reactive, toxic melanin precursors out of the melanosome. Intracellular Pmel17 amyloidogenesis is carefully orchestrated by the secretory pathway, utilizing membrane sequestration and proteolytic steps to protect the cell from amyloid and amyloidogenic intermediates that can be toxic. While functional and pathological amyloid share similar structural features, critical differences in packaging and kinetics of assembly enable the usage of Pmel17 amyloid for normal function. The discovery of native Pmel17 amyloid in mammals provides key insight into the molecular basis of both melanin formation and amyloid pathology, and demonstrates that native amyloid (amyloidin) may be an ancient, evolutionarily conserved protein quaternary structure underpinning diverse pathways contributing to normal cell and tissue physiology.

- Granata A, Monzani E, Bubacco L, Casella L.
Mechanistic insight into the activity of tyrosinase from variable-temperature studies in an aqueous/organic solvent. Chemistry. 12(9):2504-2514, 2006.
The activity of mushroom tyrosinase towards a representative series of phenolic and diphenolic substrates structurally related to tyrosine has been investigated in a mixed solvent of 34.4% methanol-glycerol (7:1, v/v) and 65.6% (v/v) aqueous 50 mM Hepes buffer at pH 6.8 at various temperatures. The kinetic activation parameters controlling the enzymatic reactions and the thermodynamic parameters associated with the process of substrate binding to the enzyme active species have been deduced from the temperature variation of the kcat and KM parameters.The activation free energy is dominated by the enthalpic term, the value of which lies in the relatively narrow range of 61+/-9 kJ mol(-1) irrespective of substrate or reaction type (monophenolase or diphenolase). The activation entropies are small and generally negative and contribute no more than 10% to the activation free energy. The substrate binding parameters are characterized by large and negative enthalpy and entropy contributions, which are typically dictated by polar protein-substrate interactions. The substrate 4-hydroxyphenylpropionic acid exhibits a strikingly anomalous temperatura dependence of the enzymatic oxidation rate, with deltaH(double dagger) approximately = 150 kJ mol(-1) and deltaS(double dagger) approximately = 280 J K(-1) mol(-1), due to the fact that it can competitively bind to the

enzyme through the phenol group, like the other substrates, or the carboxylate group, like carboxylic acid inhibitors. A kinetic model that takes into account the dual substrate/inhibitor nature of this compound enables rationalization of this anomalous behavior.

- Hashimoto Y, Ito Y, Kato T, Motokawa T, Katagiri T, Itoh M.
Expression profiles of melanogenesis-related genes and proteins in acquired melanocytic nevus. J Cutan Pathol. 33(3):207-15, 2006.
We used three types of AMN to investigate the expression profiles of melanogenesis-related genes [tyrosinase, tyrosinase-related protein-1 (TRP1), dopachrome tautomerase (TRP2), Pmel-17/gp100, P-protein, and microphthalmia-associated transcription factor (MITF)], as well as tyrosinase, TRP1, Pmel-17/gp100, and MITF proteins. Results: All melanogenesis-related genes examined in the junctional type were expressed in the basal epidermal layer. In the compound and intradermal types, mRNA for tyrosinase, TRP2, and MITF was expressed in all of the AMN cells. However, the expression of TRP1, P-protein, and Pmel-17/gp100 in the compound type and TRP1 in the intradermal type became weaker in accordance with the depth of the dermis layer, as compared to those levels in the basal to upper dermis layer. Although tyrosinase and Pmel-17/gp100 mRNA in the compound and intradermal types was expressed in the intraepidermal and dermal components, immunohistochemical staining showed that tyrosinase proteins were not detected in the lower dermis layer and Pmel-17/gp100 proteins were not detected in the dermis. Conclusions: Our results suggest that all nevus cells that constitute AMN tissue originate from melanocytes. Further, there may be differences in the transcription levels of melanogenesis-related genes as well as in their post-transcriptional regulation between nevus cells located in the basal epidermal to upper dermis layer and those in the lower dermis layer.

- Hernandez-Romero D, Sanchez-Amat A, Solano F.
A tyrosinase with an abnormally high tyrosine hydroxylase/dopa oxidase ratio. FEBS J. 273(2):257-70, 2006.
The sequencing of the genome of Ralstonia solanacearum[Salanoubat M, Genin S, Artiguenave F, et al. (2002) Nature 415, 497-502] revealed several genes that putatively code for polyphenol oxidases (PPOs). This soil-borne pathogenic bacterium withers a wide range of plants. We detected the expression of two PPO genes (accession numbers NP_518458 and NP_519622) with high similarity to tyrosinases, both containing the six conserved histidines required to bind the pair of type-3 copper ions at the active site. Generation of null mutants in those genes by homologous recombination mutagenesis and protein purification allowed us to correlate each gene with its enzymatic activity. In contrast with all tyrosinases so far studied, the enzyme NP_518458 shows higher monophenolase than o-diphenolase activity and its initial activity does not depend on the presence of l-dopa cofactor. On the other hand, protein NP_519622 is an enzyme with a clear preference to oxidize o-diphenols and only residual monophenolase activity, behaving as a catechol oxidase. These catalytic characteristics are discussed in relation to two other characteristics apart from the six conserved histidines. One is the putative presence of a seventh histidine which interacts with the carboxy group on the substrate and controls the preference for carboxylated and decarboxylated substrates. The second is the size of the residue isosteric with the aromatic F261 reported in sweet potato catechol oxidase which acts as a gate to control accessibility to CuA at the active site.

- Kim D, Park J, Kim J, Han C, Yoon J, Kim N, Seo J, Lee C.
Flavonoids as mushroom tyrosinase inhibitors: a fluorescence quenching study. J Agric Food Chem. 54(3):935-41, 2006.
Flavonoids, a group of naturally occurring antioxidants and metal chelators, can be used as tyrosinase inhibitors due to their formation of copper-flavonoid complexes. Thus, to investigate the underlying inhibition mechanism, a large group of flavonoids from several major flavones and flavonols were tested using fluorescence quenching spectroscopy. In addition, large differences in the tyrosinase inhibitory activities and chelating capacities according to the location of the hydroxyl group(s) in combination with the A and B rings in the flavonoids were confirmed. Accordingly, the major conclusions from this work are as follows: (i) The tyrosinase inhibitory activity is not only dependent on the number of hydroxyl groups in the flavonoids, (ii) the enzyme is primarily quenched by the hydroxyl group(s) of A and B rings on the ether side of the flavonoids, and (iii) the tyrosinase inhibitory activity of 7,8,3',4'-tetrahydroxyflavone is supported by a virtual model of docking with the mushroom tyrosinase, which depicts the quenching of the enzyme. The results also demonstrated that the dihydroxy substitutions in the A and B rings are crucial for Cu2+-chelate formation, thereby influencing the tyrosinase inhibitory activity.

- Kim DS, Park SH, Kwon SB, Park ES, Huh CH, Youn SW, Park KC.
Sphingosylphosphorylcholine-induced ERK activation inhibits melanin synthesis in human melanocytes. Pigment Cell Res. 19(2):146-53, 2006.
Sphingosylphosphorylcholine (SPC) is emerging as a potent signaling-lipid mediator. In this study, we investigated the effects of SPC on melanogenesis using cultured human melanocytes. Our results show that SPC significantly inhibits melanin synthesis in a concentration-dependent manner, and further that it reduces the activity of tyrosinase, the rate-limiting melanogenic enzyme. SPC treatment was also found to induce short-thick dendrites in human melanocytes, but not to reduce tyrosinase activity in a cell-free system, whereas kojic acid directly inhibited tyrosinase. These results suggest that SPC reduces pigmentation by indirectly regulating tyrosinase. In further experiments, SPC was found to downregulate microphthalmia-associated transcription factor (MITF) and tyrosinase, and Western blotting showed that SPC induces the activations of extracellular signal-regulated kinase (ERK) and 90 kDa ribosomal S6 kinase (RSK-1).

Moreover, the specific ERK pathway inhibitor, PD98059, blocked the hypopigmentation effect of SPC, and abrogated the SPC-mediated downregulation of MITF. These results suggest that the ERK pathway is involved in the melanogenic signaling cascade, and that ERK activation by SPC reduces melanin synthesis via MITF downregulation.

- Kim KS, Kim JA, Eom SY, Lee SH, Min KR, Kim Y.
Inhibitory effect of piperlonguminine on melanin production in melanoma B16 cell line by downregulation of tyrosinase expression. Pigment Cell Res. 19(1):90-8, 2006.
Tyrosinase is a key enzyme for melanin biosynthesis, and hyperpigmentation disorders are associated with abnormal accumulation of melanin pigments, which can be improved by treatment with depigmenting agents. In the present study, piperlonguminine from Piper longum was discovered to inhibit melanin production in melanoma B16 cells stimulated with alpha-melanocyte stimulating hormone (alpha-MSH), 3-isobutyl-1-methylxanthine or protoporphyrin IX, where the compound exhibited stronger depigmenting efficacy than kojic acid. However, piperlonguminine did not affect 1-oleoyl-2-acetyl-sn-glycerol-induced melanogenesis and did not affect protein kinase C-mediated melanin production. Surprisingly, piperlonguminine did not inhibit the catalytic activity of cell-free tyrosinase from melanoma B16 cells but rather suppressed tyrosinase mRNA expression. This effect was attributed to the inhibitory action of piperlonguminine on alpha-MSH-induced signaling through cAMP to the cAMP responsive element binding protein that in turn regulates the expression of the microphthalmia-associated transcription factor, a key activator of the tyrosinase promoter. This study demonstrates that piperlonguminine is an efficient depigmenting agent with a novel mechanism of action.

- Lavado A, Jeffery G, Tovar V, de la Villa P, Montoliu L.
Ectopic expression of tyrosine hydroxylase in the pigmented epithelium rescues the retinal abnormalities and visual function common in albinos in the absence of melanin. J Neurochem. 96(4):1201-11, 2006.
Albino mammals have profound retinal abnormalities, including photoreceptor deficits and misrouted hemispheric pathways into the brain, demonstrating that melanin or its precursors are required for normal retinal development. Tyrosinase, the primary enzyme in melanin synthesis commonly mutated in albinism, oxidizes l-tyrosine to l-dopaquinone using l-3,4-dihydroxyphenylalanine (L-DOPA) as an intermediate product. L-DOPA is known to signal cell cycle exit during retinal development and plays an important role in the regulation of retinal development. Here, we have mimicked L-DOPA production by ectopically expressing tyrosine hydroxylase in mouse albino retinal pigment epithelium cells. Tyrosine hydroxylase can only oxidize l-tyrosine to L-DOPA without further progression towards melanin. The resulting transgenic animals remain phenotypically albino, but their visual abnormalities are corrected, with normal photoreceptor numbers and hemispheric pathways and improved visual function, assessed by an increase of spatial acuity. Our results demonstrate definitively that only early melanin precursors, L-DOPA or its metabolic derivatives, are vital in the appropriate development of mammalian retinae. They further highlight the value of substituting independent but biochemically related enzymes to overcome developmental abnormalities.

- Lewandowski AT, Small DA, Chen T, Payne GF, Bentley WE.
Tyrosine-based "Activatable Pro-Tag": Enzyme-catalyzed protein capture and release. Biotechnol Bioeng.93(6):1207-15, 2006.
Protein recovery is often achieved by a series of capture and release steps that often involve chromatographic binding and elution. We report an alternative, non-chromatographic, capture and release approach that employs enzymes and the stimuli-responsive polysaccharide chitosan. We capture our protein using the enzyme tyrosinase that oxidizes accessible tyrosine residues of the protein and "activates" these residues for covalent capture (i.e., conjugation) onto chitosan. Using fusions of green fluorescent protein (GFP) we observed that: (i) enzymatic activation is required for protein capture to chitosan; and (ii) capture is enhanced (approximately five-fold) by engineering the protein to have a penta-tyrosine fusion tag that provides additional accessible tyrosine residues for enzymatic activation. Because the fusion tag appears to be the primary site for capture, and capture requires activation, we designate penta-tyrosine as a "pro-tag." The captured GFP-chitosan conjugate possesses the pH-responsive solubility that is characteristic of chitosan. We exploit this pH-responsive solubility to facilitate purification of the captured protein. Two enzymatic methods were explored to release the captured GFP from the chitosan conjugate. The first method employs enterokinase (EK) to cleave the protein at an engineered EK-cleavage site. The second method employs chitosanase to hydrolyze the chitosan backbone. Using GFP as a model protein, we demonstrated that enzymatic capture and release provides a simple, non-chromatographic means to recover proteins directly from cell lysates.

- Li B, Huang Y, Paskewitz SM.
Hen egg white lysozyme as an inhibitor of mushroom tyrosinase. FEBS Lett. 580(7):1877-82, 2006. Epub 2006 Feb 28.
We report a kinetics study on hen egg white lysozyme's (HEWL) inhibitory effect on mushroom tyrosinase catalysis of 3-(3,4-dihydroxyphenyl)-l-alanine (l-DOPA) or l-tyrosine. For the first time, we demonstrate HEWL as a robust inhibitor against mushroom tyrosinase in catalysis of both substrates. The kinetics pattern matches a mixed (mostly non-competitive) partial inhibition. K(i) and ID(50) value of HEWL are more than 20-fold lower than that of kojic acid, a well-known chemical inhibitor of mushroom tyrosinase. K(i), alpha value and beta value, are almost identical in both experiments (l-DOPA and l-tyrosine as substrates, respectively), which suggests this common inhibition mechanism affects both steps. The inhibitory effect increases as both proteins were mixed and pre-incubated for less

than 1h. HEWL-depletion only removed about half of the inhibitory effect. Here we propose a novel function of HEWL, which combines the reversible inhibition and the irreversible inactivation toward mushroom tyrosinase. Discovery of HEWL as an inhibitor to mushroom tyrosinase catálisis may be commercially valuable in the food, medical and cosmetic industries.

- Maresca V, Flori E, Cardinali G, Briganti S, Lombardi D, Mileo A, Paggi MG, Picardo M.
Ferritin light chain down-modulation generates depigmentation in human metastatic melanoma cells by influencing tyrosinase maturation. J Cell Physiol. 206(3):843-8, 2006.
Recently, after the identification of ferritin light chain (L-ferritin) gene and protein over-expression in human metastatic melanoma cells, we engineered, starting from the LM metastatic melanoma cell line, clones in which L-ferritin gene expression was down-regulated by the stable expression of a specific antisense construct. The present investigation started from the observation that L-ferritin down-regulated LM cells displayed a less pigmented phenotype, confirmed by a major decrease of total melanin, when compared to control LM cells. This finding was accompanied by a dramatic decrease in tyrosinase activity, which was not paralleled by a concomitant reduction of the amount of tyrosinase specific mRNA. Western blot analysis of tyrosinase in control LM cells displayed a pattern, which corresponds to the progressive glycosylation of the native protein up to the 80 kDa form, considered the functional one. Tyrosinase pattern assayed in L-ferritin down-regulated LM cells showed the remarkable absence of the 80 kDa form and a prevalence of endoglycosidase H (endo H)-sensitive immature (70 kDa) tyrosinase, accumulated in the endoplasmic reticulum (ER), as confirmed by confocal microscopy analysis. These results demonstrate that, in a human metastatic melanoma cell line, the stress condition promoted by L-ferritin down-modulation, can substantially influence proper maturation of tyrosinase.

- Matoba Y, Kumagai T, Yamamoto A, Yoshitsu H, Sugiyama M.
Crystallographic Evidence That the Dinuclear Copper Center of Tyrosinase Is Flexible during Catalysis. J Biol Chem. 281(13):8981-90, 2006.
At high resolution, we determined the crystal structures of copper-bound and metal-free tyrosinase in a complex with ORF378 designated as a "caddie" protein because it assists with transportation of two Cu(II) ions into the tyrosinase catalytic center. These structures suggest that the caddie protein covers the hydrophobic molecular surface of tyrosinase and interferes with the binding of a substrate tyrosine to the catalytic site of tyrosinase. The caddie protein, which consists of one six-strandedbeta-sheet and one alpha-helix, has no similarity with all proteins deposited into the Protein Data Bank. Although tyrosinase and catechol oxidase are classified into the type 3 copper protein family, the latter enzyme lacks monooxygenase activity. The difference in catalytic activity is based on the structural observations that a large vacant space is present just above the active center of tyrosinase and that one of the six His ligands for the two copper ions is highly flexible. These structural characteristics of tyrosinase suggest that, in the reaction that catalyzes the ortho-hydroxylation of monophenol, one of the two Cu(II) ions is coordinated by the peroxide-originated oxygen bound to the substrate. Our crystallographic study shows evidence that the tyrosinase active center formed by dinuclear coppers is flexible during catalysis.

- Mirica LM, Rudd DJ, Vance MA, Solomon EI, Hodgson KO, Hedman B, Stack TD.
mu-eta2:eta2-peroxodicopper(II) complex with a secondary diamine ligand: a functional model of tyrosinase. J Am Chem Soc. 128(8):2654-65, 2006.
The activation of dioxygen (O(2)) by Cu(I) complexes is an important process in biological systems and industrial applications. In tyrosinase, a binuclear copper enzyme, a mu-eta(2):eta(2)-peroxodicopper(II) species is accepted generally to be the active oxidant. Reported here is the characterization and reactivity of a mu-eta(2):eta(2)-peroxodicopper(II) complex synthesized by reacting the Cu(I) complex of the secondary diamine ligand N,N'-di-tert-butyl-ethylenediamine (DBED), [(DBED)Cu(MeCN)](X) (1.X, X = CF(3)SO(3)(-), CH(3)SO(3)(-), SbF(6)(-), BF(4)(-)), with O(2) at 193 K to give [[Cu(DBED)](2)(O(2))](X)(2) (2.X(2)). The UV-vis and resonance Raman spectroscopic features of 2 vary with the counteranion employed yet are invariant with change of solvent. These results implicate an intimate interaction of the counteranions with the Cu(2)O(2) core. Such interactions are supported further by extended X-ray absorption fine structure (EXAFS) analyses of solutions that reveal weak copper-counteranion interactions. The accessibility of the Cu(2)O(2) core to exogenous ligands such as these counteranions is manifest further in the reactivity of 2 with externally added substrates. Most notable is the hydroxylation reactivity with phenolates to give catechol and quinone products. Thus the strategy of using simple bidentate ligands at low temperatures provides not only spectroscopic models of tyrosinase but also functional models.

- Moridani MY.
Biochemical basis of 4-hydroxyanisole induced cell toxicity towards B16-F0 melanoma cells. Cancer Lett. 2006 Jan 17; [Epub ahead of print]
In the current work we investigated for the first time the biochemical basis of 4-hydroxyanisole (4-HA) induced toxicity in B16-F0 melanoma cells. It was found that dicoumarol, a diaphorase inhibitor, and 1-bromoheptane, a GSH depleting agent, increased 4-HA induced toxicity towards B16-F0 cells whereas dithiothreitol, a thiol containing agent, and ascorbic acid (AA), a reducing agent, largely prevented 4-HA toxicity. TEMPOL and pyrogallol, free radical scavengers, did not significantly prevent 4-HA toxicity towards B16-F0 cells. GSH>AA>NADH prevented the o-quinone formation when 4-HA was metabolized by tyrosinase/O(2). 4-HA metabolism by horseradish

peroxidase/H(2)O(2) was prevented more effectively by AA than NADH>GSH. We therefore concluded that quinone formation was the major pathway for 4-HA induced toxicity in B16-F0 melanoma cells whereas free radical formation played a negligible role in the 4-HA induced toxicity.

- Munoz JL, Garcia-Molina F, Varon R, Rodriguez-Lopez JN, Garcia-Canovas F, Tudela J.
Calculating molar absorptivities for quinones: Application to the measurement of tyrosinase activity. Anal Biochem. 351(1):128-38, 2006. Epub 2006 Jan 26.
The molar absorptivities of the quinones produced from different o-diphenols, triphenols, and flavonoids were calculated by generating the respective quinines through oxidation with an excess of periodate. Oxidation of these substrates by this reagent was analogous to oxidation by tyrosinase with molecular oxygen, although the procedure showed several advantages over the enzymatic method in that oxidation took place almost immediately and quinone stability was favored because no substrate remained. The o-diphenols studied were pyrocatechol, 4-methylcatechol, 4-tert-butylcatechol, 3,4-dihydroxyphenylalanine, 3,4-dihydroxyphenylethylamine, 3,4-dihydroxyphenylacetic acid, 3,4-dihydroxyphenylpropionic acid, and caffeic acid; the triphenols studied were pyrogallol, 1,2,4-benzenetriol, 6-hydroxydopa, and 6-hydroxydopamine; and the flavonoids studied were (+)catechin, (-)epicatechin, and quercetin. In addition, the stability of the quinones generated by oxidation of the compounds by [periodate](0)/[substrate](0)<<1 was studied. Taking the findings into account, tyrosinase could be measured by following o-quinone formation in rapid kinetic studies using the stopped-flow method. However, measuring o-quinone formation could not be useful for steady-state studies. Therefore, several methods for following tyrosinase activity are proposed, and a kinetic characterization of the enzyme's action on these substrates is made.

- Murisier F, Beermann F.
Genetics of pigment cells: lessons from the tyrosinase gene family. Histol Histopathol. 21(5):567-78, 2006.
In mammals, the melanin pigment is produced in two cell types of distinct developmental origins. The melanocytes of the skin originate form the neural crest whereas the retinal pigment epithelium (RPE) of the eye originates from the optic cup. The genetic programs governing these two cell types are tus quite different but have evolved to allow the expression of pigment cell-specific genes such as the three members of the tyrosinase-related family. Tyrosinase, Tyrp1 and Dct promoters contain a motif termed E-box which is bound by the transcription factor Mitf. These E-boxes are also found in the promoters of the corresponding fish genes, thus highlighting the pivotal role of Mitf in pigment cell-specific gene regulation. Mitf, which displays cell type-specific isoforms, transactivates the promoters of the tyrosinase gene family in both pigment cell lineages. However, specific DNA motifs have been found in these promoters, and they correspond to binding sites for RPE-specific factors such as Otx2 or for melanocyte-specific factors such as Sox10 or Pax3. The regulation of pigment cell-specific expression is also controlled by genetic elements located outside of the promoter, such as the tyrosinase distal regulatory element located at -15 kb which acts as a melanocyte-specific enhancer but also protects from spreading of condensed chromatin. Thus, by using the tyrosinase gene family as a model, it is possible to define the transcription factor networks that govern pigment production in either melanocytes or RPE.

- Ni-Komatsu L, Orlow SJ.
Heterologous expression of tyrosinase recapitulates the misprocessing and mistrafficking in oculocutaneous albinism type 2: effects of altering intracellular pH and pink-eyed dilution gene expression. Exp Eye Res. 82(3):519-28, 2006. Epub 2005 Sep 30.
The processing and trafficking of tyrosinase, a melanosomal protein essential for pigmentation, was investigated in a human epithelial 293 cell line that stably expresses the protein. The effects of the pink-eyed dilution (p) gene product, in which mutations result in oculocutaneous albinism type 2 (OCA2), on the processing and trafficking of tyrosinase in this cell line were studied. The majority of tyrosinase was retained in the endoplasmic reticulum-Golgi intermediate compartment and the early Golgi compartment in the 293 cells expressing the protein. Coexpression of p could partially correct the mistrafficking of tyrosinase in 293 cells. Tyrosinase was targeted to the late endosomal and lysosomal compartments after treatment of the cells with compounds that correct the tyrosinase mistrafficking in albino melanocytes, most likely through altering intracellular pH, while the substrate tyrosine had no effect on the processing of tyrosinase. Remarkably, this heterologous expression system recapitulates the defective processing and mistrafficking of tyrosinase observed in OCA2 albino melanocytes and certain amelanotic melanoma cells. Coexpression of other melanosomal proteins in this heterologous system may further aid our understanding of the details of normal and pathologic processing of melanosomal proteins.

- Ohguchi K, Akao Y, Nozawa Y.
Involvement of calpain in melanogenesis of mouse B16 melanoma cells. Mol Cell Biochem. 275(1-2):103-7, 2005.
In the current study, the involvement of calpain, a cysteine proteinase in the regulation of melanogenesis was examined using mouse B16 melanoma cells. In response to alpha-melanocyte-stimulating hormone (a-MSH), B16 melanoma cells underwent differentiation characterized by increased melanin biosynthesis. The total calapain activity was decreased within 2 h following alpha-MSH-treatment, and restored to the initial level in 6-12 h. To further investigate the involvement of calpain in the regulation of melanogenesis, the effect of calpain inhibitors on alpha-MSH-induced melanogenesis was examined. Inhibition of calpain by either N-acetyl-Leu-Leu-norleucinal (ALLN) or calpastatin (CS) peptide blocked alpha-MSH-induced melanogenesis. The magnitude of inhibition of melanin biosynthesis was

well correlated with a decrease in the activity of tyrosinase, a key regulatory enzyme in melanogenesis. Treatment of B16 cells with ALLN caused marked decrease in both tyrosinase protein and mRNA levels. These results indicate that calpain would be involved in the melanogenic signaling by modulating the expression of tyrosinase in mouse B16 melanoma cells.

- Okombi S, Rival D, Bonnet S, Mariotte AM, Perrier E, Boumendjel A.
Discovery of benzylidenebenzofuran-3(2H)-one (aurones) as inhibitors of tyrosinase derived from human melanocytes. J Med Chem. 49(1):329-33, 2006.
Tyrosinase is a copper-dependent enzyme which converts l- tyrosine to dopaquinone and is involved in different biological processes such as melanogenesis and skin hyperpigmentation. The purpose of this study was to investigate naturally occurring aurones (Z-benzylidenebenzofuran-3(2H)-one) and analogues as human tyrosinase inhibitors. Several aurones bearing hydroxyl groups on A-ring and different substituents on B-ring were synthesized and evaluated as inhibitors of human melanocyte-tyrosinase by an assay which measures tyrosinase-catalyzed l-Dopa oxidation. We found that unsubstituted aurones were weak inhibitors; however, derivatives with two or three hydroxyl groups preferably at 4,6 and 4' positions are able to induce significant tyrosinase inhibition. The most potent aurone was found to be the naturally occurring 4,6,4'-trihydroxyaurone which induces 75% inhibition at 0.1 mM concentration and is highly effective when compared to kojic acid, one of the best tyrosinase inhibitors known so far (the latter is completely inactive at such concentrations). Active aurones are devoid of toxic effects as shown by in vivo studies.

- Palavicini S, Granata A, Monzani E, Casella L.
Hydroxylation of phenolic compounds by a peroxodicopper(II) complex: further insight into the mechanism of tyrosinase. J Am Chem Soc. 127(51):18031-6, 2005.
The dicopper(I) complex [Cu2(MeL66)]2+ (where MeL66 is the hexadentate ligand 3,5-bis-{bis-[2-(1-methyl-1H-benzimidazol-2-yl)-ethyl]-amino}-meth ylbenzene) reacts reversibly with dioxygen at low temperature to form a mu-peroxo adduct. Kinetic studies of O2 binding carried out in acetone in the temperature range from -80 to -55 degrees C yielded the activation parameters DeltaH1(not equal) = 40.4 +/- 2.2 kJ mol(-1), DeltaS1)(not equal) = -41.4 +/- 10.8 J K(-1) mol(-1) and DeltaH(-1)(not equal) = 72.5 +/- 2.4 kJ mol(-1), DeltaS(-1)(not equal) = 46.7 +/- 11.1 J K(-1) mol(-1) for the forward and reverse reaction, respectively, and the binding parameters of O2 DeltaH degrees = -32.2 +/- 2.2 kJ mol(-1) and DeltaS degrees = -88.1 +/- 10.7 J K(-1) mol(-1). The hydroxylation of a series of p-substituted phenolate salts by [Cu2(MeL66)O2]2+ studied in acetone at -55 degrees C indicates that the reaction occurs with an electrophilic aromatic substitution mechanism, with a Hammett constant rho = -1.84. The temperature dependence of the phenol hydroxylation was studied between -84 and -70 degrees C for a range of sodium p-cyanophenolate concentrations. The rate plots were hyperbolic and enabled to derive the activation parameters for the monophenolase reaction DeltaH(not equal)ox = 29.1 +/- 3.0 kJ mol(-1), DeltaS(not equal)ox = -115 +/- 15 J K(-1) mol(-1), and the binding parameters of the phenolate to the mu-peroxo species DeltaH degrees(b) = -8.1 +/- 1.2 kJ mol(-1) and DeltaS degrees(b) = -8.9 +/- 6.2 J K(-1) mol(-1). Thus, the complete set of kinetic and thermodynamic parameters for the two separate steps of O2 binding and phenol hydroxylation have been obtained for [Cu2(MeL66)]2+.

- Park HY, Wu C, Yonemoto L, Murphy-Smith M, Wu H, Stachur CM, Gilchrest BA.
MITF mediates cAMP-induced PKC-beta expression in human melanocytes. Biochem J. 2006 Jan 17; [Epub ahead of print]
The cAMP-dependent pathway up-regulates the microphthalmia-associated transcription factor (MITF), important for key melanogenic proteins such as tyrosinase, TRP-1 and TRP-2. We asked whether MITF is also a key transcription factor for protein kinase C-beta (PKC-beta), required to phosphorylate otherwise inactive tyrosinase. When paired cultures of human melanocytes were treated with IBMX, known to increase intracellular cAMP, both protein and mRNA levels of PKC-beta were induced by 24 hours. To determine whether MITF modulates PKC-beta expression, paired cultures of human melanocytes were transfected with dominant negative MITF (dn-MITF) or empty control vector. By immunoblotting, PKC-beta protein was reduced by 63 +/- 3.7% within 48 hours. Co-transfection of an expression vector for MITF-M, the MITF isoform specific for pigment cells, or empty control vector with a full-length PKC-beta promoter-CAT reporter construct (PKC-beta/CAT) into Cos-7 cells showed a > 60-fold increase in CAT activity. Melanocytes abundantly also expressed MITF-A, as well as the -B and -H isoforms. However, in contrast to MITF-M, MITF-A failed to transactivate co-expressed PKC-beta/CAT or CAT constructs under control of a full-length tyrosinase promoter. Together these results demonstrate that MITF, specifically MITF-M, is a key transcription factor for PKC-beta, linking the PKC and cAMP-dependent pathways in regulation of melanogenesis.

- Salas-Cortes L, Ye F, Tenza D, Wilhelm C, Theos A, Louvard D, Raposo G, Coudrier E.
Myosin Ib modulates the morphology and the protein transport within multi-vesicular sorting endosomes. J Cell Sci. 118(Pt 20):4823-32, 2005.
Members of at least four classes of myosin (I, II, V and VI) have been implicated in the dynamics of a large variety of organelles. Despite their common motor domain structure, some of these myosins, however, are non processive and cannot move organelles along the actin tracks. Here, we demonstrate in the human pigmented MNT-1 cell line that, (1) the overexpression of one of these myosins, myosin 1b, or the addition of cytochalasin D affects the morphology of the sorting multivesicular endosomes; (2) the overexpression of myosin 1b delays the processing of Pmel17 (the product of murine silver locus also named GP100), which occurs in these multivesicular endosomes; (3) myosin 1b associated

with endosomes coimmunoprecipitates with Pmel17. All together, these observations suggest that myosin 1b controls the traffic of protein cargo in multivesicular endosomes most probably through its ability to modulate with actin the morphology of these sorting endosomes.

- Santiago Borrero P, Rodriguez-Perez Y, Renta JY, Izquierdo NJ, Del Fierro L, Munoz D, Molina N, Ramirez S, Pagan-Mercado G, Ortiz I, Rivera-Caragol E, Spritz R, Cadilla CL.
  Genetic testing for oculocutaneous albinism type 1 and 2 and Hermansky-Pudlak syndrome type 1 and 3 mutations in Puerto Rico. J Invest Dermatol. 126(1):85-90, 2006.
  Hermansky-Pudlak syndrome (HPS) (MIM #203300) is a heterogeneous group of autosomal recessive disorders characterized by oculocutaneous albinism (OCA), bleeding tendency, and lysosomal dysfunction. HPS is very common in Puerto Rico (PR), particularly in the northwest part of the island, with a frequency of approximately 1:1,800. Two HPS genes and mutations have been identified in PR, a 16-base pair (bp) duplication in HPS1 and a 3,904-bp deletion in HPS3. In Puerto Ricans with more typical OCA, the most common mutation of the tyrosinase (TYR) (human tyrosinase (OCA1) gene) gene was G47D. We describe screening 229 Puerto Rican OCA patients for these mutations, and for mutations in the OCA2 gene. We found the HPS1 mutation in 42.8% of cases, the HPS3 deletion in 17%, the TYR G47D mutation in 3.0%, and a 2.4-kb deletion of the OCA2 gene in 1.3%. Among Puerto Rican newborns, the frequency of the HPS1 mutation is highest in northwest PR (1:21; 4.8%) and lower in central PR (1:64; 1.6%). The HPS3 gene deletion is most frequent in central PR (1:32; 3.1%). Our findings provide insights into the genetics of albinism and HPS in PR, and provide the basis for genetic screening for these disorders in this minority population.

- Sarangarajan R, Apte SP.
  The polymerization of melanin: a poorly understood phenomenon with egregious biological implications. Melanoma Res. 16(1):3-10, 2006.
  Several hypotheses have explicitly implicated the role of an altered redox status of melanin in the aetiology of melanoma and macular degeneration. The balance between the intrinsic anti-oxidant and pro-oxidant properties of melanin is lost, resulting in an altered redox phenotype. We propose that such an alteration of the redox status of melanin may arise, in part, due to suboptimal conditions for the effective polymerization of melanin precursors. We suggest that a decrease in the degree of polymerization or molecular weight of the melanin polymer may cause an alteration of the redox status of the polymer towards a more pro-oxidant state. A higher propensity of smaller oligomers to complex metals, coupled with an upregulation of metallothionein expression, results in increased production of free radicals including the superoxide anion. This, in association with an increase in the rate of tyrosinase degradation, a decrease in the rate of tyrosinase activation, alterations to template protein structure or alterations in the kinetics of the oxidation of tyrosine via the Raper-Mason pathway, may result in an overcoming of the cellular anti-oxidant pool, an increased susceptibility to oxidative stress and alterations to the reaction kinetics of melanogenesis, thus setting up a cycle of increasing oxidative stress and proliferation leading to the leakage of melanin monomers outside the organelle, thereby causing cytotoxicity and necrosis.

- Sarkar C, Singh SK, Mandal SK, Saha B, Bera R, Ratha J, Datta PK, Bhadra R.
  Human placental protein/peptides stimulate melanin synthesis by enhancing tyrosinase gene expression. Mol Cell Biochem. 2006 Feb 14;:1-10 [Epub ahead of print]
  Placental protein/peptides as biological response modifier are well documented, but not much known about melanogenesis. We possibly for the first time, demonstrated melanogenesis in B16F10 mouse melanoma by a placental protein/peptide fraction (PPPF) prepared from a hydroalcoholic extract of fresh term human placenta. This study described the effect of PPPF on the induction of tyrosinase; the key enzyme of melanogenesis to investigate the basis of PPPF induced pigmentation in primary melanocyte and B16F10 melanoma. Tyrosinase induction by PPPF in B16F10 cells was found dose- and time dependent at the level of activity. Tyrosinase, at the level of transcription and protein expression when assessed by RT-PCR and Western blot analyses found to have considerable induction over untreated control. PPPF led to enhanced activation of tyrosinase promoter resulting higher transcription thus substantiating the role of PPPF as a stimulator of melanogenesis. Actinomycin D, the transcriptional inhibitor of protein synthesis, blocked the stimulatory action of PPPF since the induction of tyrosinase and melanin was markedly reduced in presence of this inhibitor. Thus the results suggested that PPPF mediated increase in tyrosinase expression occurred through transcriptional upregulation to stimulate melanogenesis in B16F10 cells and in primary melanocyte also.

- Scott GA, Jacobs SE, Pentland AP.
  sPLA(2)-X Stimulates Cutaneous Melanocyte Dendricity and Pigmentation Through a Lysophosphatidylcholine-Dependent Mechanism. J Invest Dermatol. 126(4):855-61, 2006.
  Photoprotection of the skin is provided by melanocytes, neural crest derived cells that synthesize melanin in specialized organelles that are transferred to keratinocytes. Secretory phospholipases comprise a large family of Ca(2+)-dependent enzymes that liberate arachidonic acid (AA), a precursor of prostaglandins, as well as lysophospholipids. The predominant secretory phospholipase expressed by keratinocytes is group X secretory phospholipase A(2) (sPLA(2)), which liberates large amounts of AA and the lysophospholipid lysophosphatidylcholine (LPC), from membrane preparations. Recent work by our laboratory has shown that melanocytes express receptors for

prostaglandins that upon activation stimulate melanocyte dendricity and activity of tyrosinase, a key enzyme in melanin biosynthesis. In the present study, we have treated human melanocytes with recombinant sPLA(2)-X and show that low levels of sPLA(2)-X stimulate both tyrosinase activity and melanocyte dendricity. We found that the effects of sPLA(2)-X are mediated predominantly by LPC, not AA, and we have demonstrated expression of the phospholipase A2 receptor and two G-protein-coupled receptors for LPC (G2A and GPR119) in human melanocytes. Because secretory phospholipases are released during inflammation and are regulated by UV irradiation, our data suggest an important role for sPLA(2)-X in cutaneous pigmentation through the release of LPC.

- Song KK, Huang H, Han P, Zhang CL, Shi Y, Chen QX.
Inhibitory effects of cis- and trans-isomers of 3,5-dihydroxystilbene on the activity of mushroom tyrosinase. Biochem Biophys Res Commun. 342(4):1147-51, 2006. Epub 2006 Feb 17.
The effects of cis- and trans-isomers of 3,5-dihydroxystilbene on the activity of mushroom tyrosinase have been studied. The results show that both cis- and trans-isomers of 3,5-dihydroxystilbene can inhibit the diphenolase activity of the enzyme and the inhibition type was reversible. The IC(50) values were estimated as 0.405+/-0.013 and 0.705+/-0.017mM, respectively. Kinetic análisis showed that the inhibition of cis-3,5-dihydroxystilbene and trans-3,5-dihydroxystilbene on the diphenolase activity of the enzyme belonged to competitive type, and the inhibition constants (K(I)) were determined to be 0.232+/-0.015 and 0.395+/-0.020mM, respectively. In this investigation, the inhibitory effects of cis-3,5-dihydroxystilbene and trans-3,5-dihydroxystilbene on the diphenolase activity of mushroom tyrosinase were compared. The inhibitory capacity of cis-isomer was stronger than that of corresponding trans-isomer. Nevertheless, the trans-3,5-dihydroxystilbene was used more frequently than its corresponding cis-form compound. This research may offer some references for designing and synthesizing some novel and effective tyrosinase inhibitors. Furthermore, it may improve the use of stilbenes on the field of food preservation and depigmentation.

- Theos AC, Truschel ST, Tenza D, Hurbain I, Harper DC, Berson JF, Thomas PC, Raposo G, Marks MS.
A lumenal domain-dependent pathway for sorting to intralumenal vesicles of multivesicular endosomes involved in organelle morphogenesis. Dev Cell. 10(3):343-54, 2006.
Cargo partitioning into intralumenal vesicles (ILVs) of multivesicular endosotes underlies such cellular processes as receptor downregulation, viral budding, and biogenesis of lysosome-related organelles such as melanosomes. We show that the melanosomal protein Pmel17 is sorted into ILVs by a mechanism that is dependent upon lumenal determinants and conserved in non-pigment cells. Pmel17 targeting to ILVs does not require its native cytoplasmic domain or cytoplasmic residues targeted by ubiquitylation and, unlike sorting of ubiquitylated cargo, is insensitive to functional inhibition of Hrs and ESCRT complexes. Chimeric protein and deletion analyses indicate that two N-terminal lumenal subdomains are necessary and sufficient for ILV targeting. Pmel17 fibril formation, which occurs during melanosome maturation in melanocytes, requires a third luminal subdomain and proteolytic processing that itself requires ILV localization. These results establish an Hrs- and perhaps ESCRT-independent pathway of ILV sorting by lumenal determinants and a requirement for ILV sorting in fibril formation.

- Tsuji-Naito K, Hatani T, Okada T, Tehara T.
Evidence for covalent lipoyl adduction with dopaquinone following tyrosinase-catalyzed oxidation. Biochem Biophys Res Commun. 343(1):15-20, 2006.
Previous studies have examined the conjugation of sulfhydryl compounds such as l-cysteine and glutathione with DOPA-quinone following the oxidation of tyrosine and DOPA by tyrosinase. These covalent reactions play a key role in the regulation and metabolism of pigment cells. We report on the first direct evidence for the formation of lipoyl adducts in reactions of thiol groups with DOPA-quinone in dihydrolipoic acid (6,8-dimercaptooctanoic acid [DHLA]). Incubating DHLA with DOPA-quinone followed by tyrosinase-catalyzed oxidation resulted in the three products predicted by HPLC-UV and LC-ESI(-)-MS analyses for DHLA DOPA conjugates. In the current study, we identified 5-S-lipoyl-DOPA among the principal products isolated by HPLC and characterized by FAB(-)-MS, ESI(-)-MS/MS, and (1)H NMR, 2D-COSY studies. Collectively, these results suggest that DHLA undergoes sulfhydryl conjugation with DOPA-quinone, pointing to the involvement of thiol-reactive metabolites.

- Valencia JC, Watabe H, Chi A, Rouzaud F, Chen KG, Vieira WD, Takahashi K, Yamaguchi Y, Berens W, Nagashima K, Shabanowitz J, Hunt DF, Appella E, Hearing VJ.
Sorting of Pmel17 to melanosomes through the plasma membrane by AP1 and AP2: evidence for the polarized nature of melanocytes. J Cell Sci. 119(Pt 6):1080-91, 2006.
Adaptor proteins (AP) play important roles in the sorting of proteins from the trans-Golgi network, but how they function in the sorting of various melanosome-specific proteins such as Pmel17, an essential structural component of melanosomes, in melanocytes is unknown. We characterized the processing and trafficking of Pmel17 via adaptor protein complexes within melanocytic cells. Proteomics analysis detected Pmel17, AP1 and AP2, but not AP3 or AP4 in early melanosomes. Real-time PCR, immunolabeling and tissue in-situ hybridization confirmed the coexpression of AP1 isoforms mu1A and mu1B (expressed only in polarized cells) in melanocytes and keratinocytes, but expression of mu1B is missing in some melanoma cell lines. Transfection with AP1 isoforms (mu1A or mu1B) showed two distinct distribution patterns that involved Pmel17, and only mu1B was able to restore the sorting of Pmel17 to the plasma membrane in cells lacking mu1B expression. Finally, we established that expression of mu1B is regulated physiologically in melanocytes by UV radiation or DKK1. These results show that Pmel17 is sorted to melanosomes

by various intracellular routes, directly or indirectly through the plasma membrane, and the presence of basolateral elements in melanocytes suggests their polarized nature.

- Wakamatsu K, Kavanagh R, Kadekaro AL, Terzieva S, Sturm RA, Leachman S, Abdel-Malek Z, Ito S.
  Diversity of pigmentation in cultured human melanocytes is due to differences in the type as well as quantity of melanin. Pigment Cell Res. 19(2):154-62, 2006.
  Cultured human melanocytes differ tremendously in visual pigmentation, and recapitulate the pigmentary phenotype of the donor's skin. This diversity arises from variation in type as well as quantity of melanin produced. Here, we measured contents of eumelanin (EM) and pheomelanin (PM) in 60 primary human melanocyte cultures (51 neonatal and nine adults), and correlated some of these values with the respective activity and protein levels of tyrosinase, and the melanocortin-1 receptor (MC1R) genotype. Melanocytes were classified into tour phenotypes (L, L+, D, D+) as depicted by visual pigmentation using light microscopy, and by the pigmentary phenotype of the donor's skin. There were large differences in total melanin (TM) and EM, which increased progressively for L, L+, D and D+ melanocytes. TM content, the sum of EM and PM, showed a good correlation with TM measured spectrophotometrically, and with the activity and protein levels of tyrosinase. Log EM/PM ratio did not correlate with MC1R genotype. We conclude that: (i) EM consistently correlates with the visual phenotype; (ii) lighter melanocytes tend to be more pheomelanic in composition than darker melanocytes; (iii) in adult melanocyte cultures, EM correlates with the ethnic background of the donors (African-American > Indian > Caucasian); and (iv) MC1R loss-of-function mutations do not necessarily alter the phenotype of cultured melanocytes.

- Wang N, Hebert DN.
  Tyrosinase maturation through the mammalian secretory pathway: bringing color to life. Pigment Cell Res. 19(1):3-18, 2006.
  Tyrosinase has been extensively utilized as a model substrate to study the maturation of glycoproteins in the mammalian secretory pathway. The visual nature of its enzymatic activity (melanin production) has facilitated the identification and characterization of the proteins that assist it becoming a functional enzyme, localized to its proper cellular location. Here, we review the steps involved in the maturation of tyrosinase from when it is first synthesized by cytosolic ribosomes until the mature protein reaches its post-Golgi residence in the melanosomes. These steps include protein processing, covalent modifications, chaperone binding, oligomerization, and trafficking. The disruption of any of these steps can lead to a wide range of pigmentation disorders.

- Wang R, Tang P, Wang P, Boissy RE, Zheng H.
  Regulation of tyrosinase trafficking and processing by presenilins: partial loss of function by familial Alzheimer's disease mutation. Proc Natl Acad Sci U S A. 103(2):353-8, 2006.
  Presenilins (PS) are required for gamma-secretase cleavage of multiple type I membrane proteins including the amyloid precursor protein and Notch and also have been implicated in regulating intracellular protein trafficking and turnover. Using genetic and pharmacological approaches, we reveal here a unique function of PS in the pigmentation of retinal pigment epithelium and epidermal melanocytes. PS deficiency leads to aberrant accumulation of tyrosinase (Tyr)-containing 50-nm post-Golgi vesicles that are normally destined to melanosomes. This trafficking is gamma-secretase-dependent, and abnormal localization of Tyr in the absence of PS is accompanied by the simultaneous accumulation of its C-terminal fragment. Furthermore, we show that the PS1M146V familial Alzheimer's disease mutation exhibits a partial loss-of-function in pigment synthesis. Our results identify Tyr and related proteins as physiological substrates of PS and link gamma-secretase activity with intracellular protein transport.

- Zhang C, Xie L, Huang J, Chen L, Zhang R.
  A novel putative tyrosinase involved in periostracum formation from the peral oyster (Pinctada fucata). Biochem Biophys Res Commun. 342(2):632-9, 2006.
  Tyrosinase (monophenol, L-DOPA: oxygen oxidoreductase, EC 1.14.18.1), a kind of copper-containing phenoloxidase, arouses great interests of scientists for its important role in periostracum formation. A cDNA clone encoding a putative tyrosinase, termed OT47 because of its estimated molecular mass of 47kDa, was isolated from the pearl oyster, Pinctada fucata. This novel tyrosinase shares similarity with the cephalopod tyrosinases and other type 3 copper proteins within two conserved copper-binding sites. RT-PCR analysis showed that OT47 mRNA was expressed only in the mantle edge. Further in situ hybridization analysis and tyrosinase activity staining revealed that OT47 was expressed at the outer epithelial cells of the middle fold, different from early histological results in Mercenaria mercenaria, suggesting a different model of periostracum secretion in P. fucata. Taken together, these results suggest that OT47 is most likely involved in periostracum formation. The identification and characterization of oyster tyrosinase also help to further understand the structural and functional properties of molluscan tyrosinase.

8. Melanosomes

(Prof. J. Borovansky)

It is possible to state generally that ocular, especially RPE melanosomes , have received much attention recently (*Azarian et al, Cortese et al, Eibl et al, Faraggi et al, Futter, Runkel et al, Tolleson, Tolleson et al, Zareba et al*). There are four very good reviews (*Futter, Sarangajaran & Apte, Tolleson et al, Wei*) dealing with various aspects of melanosome and melanocyte research. *Aspengren et al, De Schepper et al, Futter, Zannelli et al* have brought new information on melanosome transport. Melanosome ultrastructure was studied in spontaneous uveal melanomas in transgenic mice (*Tolleson et al*), in animals with *Grey*, a novel mutation in the murine *Lyst* gene (*Runkel et al*) and in various mouse albinism models (*Cortese et al*). An interaction – between amyloid precursor protein, neurofibromin and melanosome was observed in neurofibromatosis (*De Schepper et al*). Modern techniques have brought new information on the elemental composition of RPE and choroid melanosomes (*Eibl et al*) and on proteins in porcine RPE melanosomes (*Azarian et al*). Melanosome genesis was reported in a glioblastoma cell line (*Bonfigli et al*). Sorting and trafficking of melanosomal proteins were central topics of several papers (*Katzmann, Ni-Komatsu & Orlow, Valencia et al, Wei*). *Cortese et al* have suggested that the *OA1* gene controls both the rate of melanosome biogenesis and organelle size. *Zareba et al* have concluded that if melanosome performs a cytoprotective role within a cell, its effect may be limited to local environment of the organelle and undetectable by conventional methods.

- Aspengren S, Hedberg D, Wallin M.
  Studies of pigment transfer between *Xenopus laevis* melanophores and fibroblasts in vitro and in vivo. Pigment Cell Res 19(2): 136-145, 2006.
  Comments: Frog melanophores exist both in epidermis where keratinocytes are present and in dermis where fibroblasts dominate. Since no frog keratinocyte cell line exists, the authors studied whether release and transfer of melanosomes can be studied in a melanophore – fibroblast coculture. Evidence was found for exocytosis/endocytosis and cytophagocytosis of melanosomes. They were transferred as membrane-enclosed organelles in a process that was upregulated by α-MSH and that was different from that of latex beads. In vivo studies confirmed the presence of extracellular pigment in dermis and uptake of melanosomes by fibroblasts.

- Azarian SM, McLoad I, Lillo C, Gibbs D, Yates JR, Williams DS.
  Proteomic analysis of mature melanosomes from the retinal pigment epithelium. J Proteom Res 5(3): 521-529, 2006.
  Comments: The protein spectrum of melanosomes prepared from the porcine RPE was studied by means of mass spectrometry and 102 proteins were found including several lysosomal enzymes. The authors conclude that melanosomes may contribute to the degradation of ingested photoreceptor outer segment discs. (A modification of RPE melanosome isolation procedure was introduced. Optiprep proved to be superior to Percoll or sucrose in gradient preparation).

- Bonfigli A, Zarivi O, Colafarina S, Cimini AM, Ragnelli AM, Aimola P, Natali PG, Ceru MP, Amicarelli F, Miranda M.
  Human glioblastoma ADF cells express tyrosinase, L-tyrosine hydroxylase and melanosomes and are sensitive to L-tyrosine and phenylthiourea. J Cell Physiol 207(3): 675-682, 2006.
  Comments: The authors demonstrated that glioblastoma cells possessed tyrosinase as well as tyrosine hydroxylase activities and synthesized melanosomes. They also found that L-tyrosine downregulated the expression of the peroxisomal proliferators activated receptor α transcription factor expression in glioblastoma ADF cells and induced (similarly to phenylthiourea) apoptosis in glioblastoma and neuroblastoma cells.

- Cortese K, Giordano F, Surace EM, Venturi C, Ballabio A, Tacchetti C, Marigo V.
  The ocular albinism type 1 (*OA1*) gene controls melanosome maturation and size. Invest Ophthalmol Vis Sci 46(12): 4358-4364, 2005.
  Comments: Immunohistochemical and ultrastructural study of both tyrosinase activity and OA1 protein expression in various albinism mouse models ($Oa1^{-/-}/Oa1^{-/-}$; $Tyr^{c2\text{-}J}/Tyr^{c2\text{-}J}$ and $Oa1^{-/-}$; $Matp^{uw}/Matp^{uw}$) indicated that OA1, mutated in ocular albinism type 1, controls the rate of melanosome biogenesis at early stage of organellogenesis but at later stages the size of melanosomes.

- De Schepper S, Boucneau JM, Westbroek W, Mommaas M, Onderwater J, Messiaen L, Naeyaert JM, Lambert JL.
  Neurofibromatosis type 1 protein and amyloid precursor protein interact in normal human melanocytes and colocalize with melanosomes. J Invest Dermatol 126(3): 547-550, 2006.
  Comments: A novel interaction between the amyloid precursor protein and neurofibromin (gene product of neurofibromatosis gene) was identified. In addition, a colocalization of amyloid precursor protein and neurofibromin with melanosomes was observed. Amyloid precursor protein has been proposed to function as a vesicle cargo receptor for the motor protein kinesin-1 in neurons. The authors suggest that a complex between amyloid precursor protein, neurofibromin and melanosome might be important for melanosome transport.

- Eibl O, Schultheiss S, Blitgen-Heinecke P, Schraermeyer U.
Quantitative chemical analysis of ocular melanosomes in the TEM. Micron 37(3): 262-276, 2006.
Comments: Chemical composition of melanosomes in RPE and choroid of a human, monkey and rat was analyzed by means of EDX (energy dispersion X-ray microanalysis). The presence of C, O, Na, Mg, K, Si, P, S, Cl and Ca was demonstrated. Ca was bound to oxygen rich sites in the melanin.As for the transition metals, a mole fraction ratio of less than 0.1 at.% was found for Fe, whereas the mole fractions of Zn and Cu were clearly beyond the minimum detectable mass only in the RPE melanosomes of human eye.

- Faraggi E, Gerstman BS, Sun JM.
Biophysical effects of pulsed lasers in the retina and other tissues containing strongly absorbing particles: shockwave and explosive bubble generation. J Biomed Optics 10(6): 64029-64029, 2005.

- Futter CE.
The molecular regulation of organelle transport in mammalian retinal pigment epithelial cells. Pigment Cell Res 19(2): 104-111, 2006.
Comments: A review summarizing what we know about the molecular regulation of melanosome movement and phagosome maturation within RPE cells and discussing the potential roles of defects of organelle transport in RPE in the pathogenesis of eye diseases. A contribution of mouse model studies in this respect has not been negligible.

- Katzmann DJ.
No ESCRT to the melanosome: MVB sorting without ubiquitin. Developmental Cell 10(3): 278-280, 2006.
Summary: Multivesicular bodies (MVBs) are critical for a variety of cellular functions ranging from lysosomal degradation to the budding of HIV. To date, delivery into MVBs has been dependent on the ESCRT (**E**ndosomal **S**orting **C**omplex **R**equired for **T**ransport) machinery.However, analysis of a melanosomal protein has uncovered an an alternative pathway for the MVB sorting.

- Ni-Komatsu L, Orlow SJ.
Heterologous expression of tyrosinase recapitulates the misprocessing and mistrafficking in oculocutaneous albinism type 2: Effects of altering intracellular pH and pink-eyed dilution gene expression. Exp Eye Res 82(3): 519-528, 2006.

- Runkel F, Büssow H, Seburn KL, Cox GA, Mc Vey Ward D, Kaplan J, Franz T.
***Grey*, a novel mutation in the murine *Lyst* gene, causes the *beige* phenotype by skipping of exon 25.** Mammalian Genome 17(3): 203-210, 2006.
Comments: A novel mutation in the mouse Lyst gene – $Lyst^{bg\text{-}grey}$ was analyzed both histologically and molecularly. Melanosomes of melanocytes associated with hair follicles and the choroid as well as RPE melanosomes were larger and irregularly shaped in homozygous mutants compared with those of wild animals. The grey phenotype may be caused by the absence or marked reduction of LYST protein due to its degradation as a consequence of a deletion in the area of exon 25.

- Sarangajaran R, Apte SP.
The polymerization of melanin: A poorly understood phenomena with egregious biological implications.
Melanoma Res 16(1): 3-10, 2006.
Comments: An interesting review presenting several lines of evidence to support a hypothesis that the degree of melanin polymerization may be causative or correlative to its redox status and proposing a model explaining the heterogeneity in the molecular weight and structure of melanin. A cycle of increasing oxidative stress and proliferation may lead to the leakage of melanin monomers outside the melanosome, thereby causing cytotoxicity.

- Tolleson WH.
Human melanocyte biology, toxicology and pathology. J Environ Sci Health 23(2): 105-161, 2005.
Comments: A modern review dealing with four populations of melanocytes located in the skin, eyes, inner ear and covering of the brain. A decent attention was paid to melanosome assembly, maturation and properties. Text is accompanied by 14 excellent figures.

- Tolleson WH, Doss JC, Latendresse J, Warbritton AR, Melchior Jr WB, Chin L.
Spontaneous uveal amelanotic melanoma in transgenic *Tyr-RAS+ Ink4a/Arf-/-* mice.
Arch Ophthalmol 123(8):1088-1094, 2005.
Comments: In transgenic *Tyr-RAS+ Ink4a/Arf-/-* mice spontaneous melanomas occur that arise within the choroid or ciliary body and share histopathological features typical of human uveal melanomas. As for melanosomes, electron microscopy revealed the presence of ovoid premelanosomes (of 100-400nm size) containing haphazardly arranged inner membranes and limiting membranes with defects. Some of the organelles were electron-dense.

- Valencia JC, Watabe H, Chi A, Rouzaud F, Chen KG, Vieira WD, Takahashi K, Yamaguchi Y, Berens W, Nagashima K, Shabanowitz J, Hunt DF, Appella E, Hearing VJ.

Sorting of Pmel17 to melanosomes through the plasma membrane by AP1 and AP2: evidence for the polarized nature of melanocytes. J Cell Sci 119(6): 1080-1091, 2006.
Comments: Processing and trafficking of melanosome protein Pmel17 via adaptor protein complexes within melanocytic cells was characterized. Modern proteomics and molecular biology analyses revealed that the Pmel17 is sorted from Golgi to stage I melanosomes directly or indirectly according to whether adaptor protein 1 isoform μ1B is expressed (e.g. in melanocytes) or not (e.g. in metastsasizing melanoma cells) – elegantly summarized in Fig.8. Moreover, it was shown that the expression of μ1B is regulated physiologically by UV radiation or DKK1, an inhibitor of Wnc signalling.

- Wei ML.
Hermansky-Pudlak syndrome: A disease of protein trafficking and organelle function.
Pigment Cell Res 18(1): 19-42, 2006.
Comments: An excellent review summarizing recent molecular, biochemical and cell biological findings and clinical studies. All eight human HPS subtypes are characterized in detail and corresponding mouse models are mentioned. Superb colour schemes explaining trafficking pathways in melanocytes mediated by HPS proteins and function of HPS protein complexes along the pathway of melanosome biogenesis.

- Zannolli R, Buoni S, Macucci F, Santi MM, Miracco F, Pierluigi M, Mogni M, Piomboni P, Massafra MR, Galluzzi P, Liwi W, Cuccia A, Margollicci MA, Pucci L, Sacco P, Molinelli M, Burlina AB, Swift JA, Fimiani M, Zappella M, Miracco C.
Global developmental delay, osteopenia and ectodermal defect: A new syndrome. Brain Dev 28(3): 155-161, 2006.
Comments: Skin defects in children affected by global developmental delay comprise various abnormalities – abnormal keratinocyte differentiation, sweat gland and melanocyte abnormalities. The latter displayed both morphological (reduced number and size without evident dendritic processes) and functional changes (defects of the melanosome migration into keratinocytes).

- Zareba M, Raciti MW, Henry MM.
Oxidative stress in ARPE-19 cultures: Do melanosomes confer cytoprotection? Free Rad Biol Med 40(1): 87-100, 2006.
Comments: To examine whether melanosomes can confer cytoprotection against oxidative stress induced chemically or photically in human amelanotic retinal pigment epithelium cells ARPE19, the cells were incubated with melanosomes isolated from bovine and porcine RPE and for comparison also with ozonated charcoal particles and silica particles. None of the phagocytized particles affected ARPE19 cell viability or levels of catalse and glutathione peroxidase neither any evidence was obtained to indicate that melanosomes confer a cytoprotection against oxidative stress. It is concluded that if melanosome performs a cytoprotective role within cell, its effect may be limited to local environment of the organelle and undetectable by conventional methods. (However, since the isolation procedure included sonication, temporary pH 1!? and repeated freezing and thawing, the melanosomes used, were not native according to my experience, and hence their behaviour did not have to be identical to a physiological situation).

ANNOUNCEMENTS & RELATED ACTIVITIES

Calendar of events

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

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

2006 Stress and the Skin

June 2-4, New-York City
Contact : JAH Meeting Planners International
4101 Atlantic-Brigantine Blvd., Suite 1
Brigantine, NJ 08203
Phone: (609) 266-2547
Fax: (609) 266-3072
Email: jahart623@aol.com

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

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

2006 The 6th International Conference on the Adjuvant Therapy of Malignant Melanoma

June 15-17, Stockholm, Sweden
Contact : Chairman: Ulrik Ringborg, MD, Ph.D.
The Oncologic Clinic
Karolinska University Hospital
SE-171 76 Stockholm, Sweden
Phone: +46 8 517 743 00
Email: ulrik.ringborg@karolinska.se

2006 4th Summer Academy of Dermatopathology Meeting

July 31 - August 04, Graz, Austria

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

2006 36th Annual ESDR Meeting

September 7-9, Paris, France

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

2006 International Dermoscopy Course and Conference

September 7-9, Warsaw, Poland

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

2006 13th meeting of the Pan American Society for Pigment Cell Research

September 7-10, Cincinnati, Ohio, USA

Contact: Zalfa Abdel-Malek
E-mail: paspcr13@uc.edu
Congress Web page: http://www.conferencing.uc.edu/Details.asp?ConferenceID=239
Web : paspcr.med.umn.edu/

2006 Perspectives in Melanoma X

September 13-16, Amsterdam, Netherlands

Contact: Imedex
Phone: 770-751-7332
Fax: 770-751-7334
Email: meetings@imedex.com
Website: www.imedex.com

2006 6th Congress of BADV

September 14-16, Riga, Latvia

Contact: Andris Y. Rubins, MD
Riga Stadins University
Kr. Valdemara Street 76-75
Riga LV-1013, Latvia

Fax: 371-737-0395
Email: arubins@apollo.lv
Website: www.badv.lv

2006 XIIIth Meeting of the ESPCR

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

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

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

2006 XXVII Symposium of the ISDP

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

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

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

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

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 XIVth Meeting of the ESPCR

September, Bari, Italy
Organizer: Prof. Rosa Cicero

2007 21st World Congress of Dermatology

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

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

December 8-9, Toyoake City, Japan
Contact: Prof. Kazumasa Wakamatsu
Web: wwwsoc.nii.ac.jp/jspcr/

2008 20th International Pigment Cell Conference (IPCC)

May 7–12 Sapporo, Japan
Contact: Secretariat Office
Toshiharu YAMASHITA (Sapporo Medical University, Japan)
Minami 1-jo, Nishi 16-chome Chuo-ku, Sapporo, Japan 060-8543
Phone: +81-11-611-2111
Fax: +81-11-613-3739
E-mail: **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

13th MEETING OF THE EUROPEAN SOCIETY FOR PIGMENT CELL RESEARCH

Barcelona, Spain, 24-27 September 2006

REGISTRATION IS OPEN
SUBMISSION OF ABSTRACTS IS OPEN

Early registration: 1 February 2006 - 31 July 2006
Abstract submission: 1 February 2006 - 31 May 2006
Late registration: 1 August 2006 - 15 September 2006

Registration and Abstract Submission through the meeting WEB site:
http://www.cnb.uam.es/~espcr06/registration.html

NEW MEMBERS

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

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